Scientific Excellence • Resource Protection & Conservation • Benefits for Canadians
Excellence scientifique • Protection et conservation des ressources • Bénéfices aux Canad

# Proceedings of Canada-Norway Finfish Aquaculture Workshop, September 11-14, 1989

R. L. Saunders, Editor

Biological Station
St. Andrews, N. B. E0G 2X0

October 1990

## Canadian Technical Report of Fisheries and Aquatic Sciences No. 1761

Fisheries and Oceans
Pêches et Océans

Canada

## Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

## Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annual des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences 1761

October 1990

# PROCEEDINGS OF CANADA-NORWAY FINFISH AQUACULTURE WORKSHOP, SEPTEMBER 11-14, 1989

Edited by

R. L. Saunders
Department of Fisheries and Oceans
Biological Sciences Branch
Biological Station
St. Andrews, New Brunswick E0G 2X0 Canada

This is the two hundred and tenth Technical Report of
the Biological Station, St. Andrews, N. B.

91-01308/

© Minister of Supply and Services Canada 1990

Cat. No. Fs 97-6/1761 ISSN 0706-6457

Correct citation for this publication:

Saunders, R. L. (Editor). 1990. Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761: v + 177 p.

## TABLE OF CONTENTS

| | Page |
|---|---|
| Foreword | v |
| Algal blooms in the Bay of Fundy - J. L. Martin and D. J. Wildish | 1 |
| Interactions between environment and fish farming - A. Ervik and P. K. Hansen | 7 |
| Sedimentary anoxia caused by salmonid mariculture wastes in the Bay of Fundy and its effects on dissolved oxygen in seawater - D. J. Wildish, V. Zitko, H. M. Akagi and A. J. Wilson | 11 |
| Vibriosis in Norwegian aquaculture - B. Hjeltnes and O. M. Rødseth | 19 |
| An overview of the current health status of cultured Atlantic salmon in the Atlantic provinces of Canada - J. W. Cornick | 25 |
| Detection of pathogens in sub-clinically and clinically infected salmonids - R. Lallier, L. LeBlanc, M. Desbiens and G. Ouellet | 31 |
| Genetic studies on farmed fish: genotype x environment interaction and genetic effects on native gene pools - K. E. Jørstad | 41 |
| An overview of selection practices in the Salmon Genetics Research Program - G. W. Friars, J. K. Bailey and F. M. O'Flynn | 49 |
| Tank effects as confounding factors in genetic experiments: experiences with Arctic charr (*Salvelinus alpinus*) - B. G. E. de March | 61 |
| Artificial hatching substrates improve growth and yolk absorption of salmonids - T. Hansen, R. Christiansen, R. Nortvedt, S. Stefansson and G. L. Taranger | 69 |
| The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*) - J. C. Holm, T. Refstie and S. Bø | 77 |
| The influence of light on growth and smolting of Atlantic salmon (*Salmo salar*): effects of spectral composition, intensity and photoperiod - S. O. Stefansson, T. Hansen and G. L. Taranger | 79 |
| Sea lice infestation on farmed salmon: possible use of cleaner-fish as an alternative method for de-lousing - Å. Bjordal | 85 |
| Activities in nutritional research at the Institute of Marine Research, Division of Aquaculture - O. J. Torrissen and I. Opstad | 91 |
| Control of the timing of smoltification in Atlantic salmon: endogenous rhythms and environmental factors - J. Duston and R. L. Saunders | 99 |
| Production of juvenile Atlantic salmon (*Salmo salar*) at the Mactaquac accelerated rearing facility, New Brunswick, Canada - G. J. Farmer, P. D. Hubley, H. Jansen, J. W. McAskill and G. B. Robbins | 107 |
| Important considerations in the mariculture of Arctic charr (*Salvelinus alpinus* L.) - J. L. Delabbio, A. Sreedharan, and B. D. Glebe | 119 |

The role of growth hormone in the adaptability of Atlantic salmon (*Salmo salar*) to seawater - G. Boeuf, A. Le Roux, A. Severe, P. Prunet and P. Y. Le Bail 125

Salmonid growth under different environmental conditions: toward a general growth model for chinook salmon - H. Kreiberg 133

Extensive startfeeding of marine fry - K. Naas 137

Cod enhancement experiments in Norway - T. Svåsand 143

Intensive production of halibut fry - A. Mangor-Jensen, T. Harboe, S. Tuene, K. Boxaspen and L. Skjolddal 153

The Atlantic halibut yolk sac larva: a summary of the results from four years of experimentation - K. Pittman 161

Response of plasma sodium, chloride and osmotic concentrations in Atlantic cod (*Gadus morhua*) following direct transfer to diluted seawater - J.-D. Dutil, M. Besner and J. Munro 169

Workshop Attendance List 175

## FOREWORD

During an extended visit to Norway in 1988, I discussed common interests in aquaculture research in Norway and Canada with Dr. Snorre Tilseth, Chief of the Aquaculture Division of the Institute of Marine Research in Bergen. He stressed that increased communication between aquaculture scientists in his country and those in eastern Canada would be advantageous to the aquaculture industries in both countries who not only work with the same species in most cases, but also face the same or similar biological problems in relation to husbandry, nutrition, disease, and genetics. Such communication would enhance the already free exchange of research findings between our two countries. Further discussion between Dr. Tilseth and Dr. Mike Sinclair, Director of Biological Sciences Branch, Scotia-Fundy Region, Department of Fisheries and Oceans, Halifax, Nova Scotia, led to a decision to hold a workshop on finfish aquaculture at the St. Andrews Biological Station under the aegis of the recently negotiated Canada-Norway Bilateral Science and Technology Agreement. I was asked to convene and chair a meeting with Canadian participants mainly from the Atlantic area, but with representation from other regions in Canada. Thirteen Norwegians, 35 Canadians, and one French scientist participated in the workshop (see attendance list). Canadian participants were mainly researchers from Department of Fisheries and Oceans, but included biologists from the university, provincial government, and private sectors. The Norwegian participants were federal government and university researchers. There were 28 verbal presentations dealing with the algal bloom menace and other ecological concerns, disease problems, nutrition research, behavioral interaction, control of parasites, salmonid husbandry, and studies with new aquaculture species. Twenty-four papers are included in these Proceedings which, I believe, are a testimony to the usefulness of the Workshop and the ongoing exchange which it has helped to generate.

## PRÉFACE

Pendant une visite prolongée que j'ai faite en Norvège en 1988, j'ai eu l'occasion de discuter des intérêts aquicoles communs entre le Canada et la Norvège, avec M. Snorre Tilseth, chef de la Division de l'aquaculture de l'Institut de recherches marines à Bergen. M. Tilseth affirmait qu'une communication accrue entre les scientifiques en aquiculture de son pays et ceux de l'est du Canada serait avantageuse pour les industries aquicoles des deux pays, puisqu'elles élèvent les mêmes espèces, ou presque, et qu'elles font face aux mêmes problèmes biologiques ou à des problèmes semblables en ce qui concerne l'élevage, la nutrition, les maladies et la génétique. Ce genre de communication accentuerait l'échange déjà fructueux de résultats de recherche d'un pays à l'autre. De plus amples discussions ont ensuite eu lieu entre M. Tilseth et M. Mike Sinclair, directeur de la Direction des sciences biologiques, région de Scotia-Fundy, ministère des Pêches et des Océans à Halifax (Nouvelle-Écosse), suite auxquelles il a été décidé d'organiser un atelier sur la culture des poissons osseux à la Station biologique de St. Andrews, sous l'égide de la récente entente bilatérale Canada-Norvège sur les sciences et la technologie. On m'a demandé de convoquer et de présider une réunion de tous les participants canadiens; la plupart proviennent des provinces de l'Atlantique mais d'autres régions du Canada y étaient aussi représentées. Ont participé à cet atelier 13 scientifiques norvégiens, 35 scientifiques canadiens et un français (voir la liste ci-annexée des personnes présentes). La délégation canadienne, composée en majeure partie de chercheurs du ministère des Pêches et des Océans, comprenait aussi des biologistes d'universités et des représentants de gouvernements provinciaux et du secteur privé. Les participants norvégiens étaient des chercheurs gouvernementaux et universitaires. Il y a eu en tout 28 présentations orales traitant de la menace des poussées d'algues et d'autres problèmes écologiques, des maladies, de la recherche en nutrition, des interactions béhaviorales, du contrôle des parasites, de la salmoniculture et des études sur les nouvelles espèces aquicoles. Le présent compte rendu des délibérations contient vingt-quatre exposés, ce qui, je pense, témoigne de l'énorme utilité de cet atelier et des échanges permanents qu'il a aidé à engendrer.

Richard L. Saunders

# ALGAL BLOOMS IN THE BAY OF FUNDY

J. L. Martin and D. J. Wildish
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick E0G 2X0
Canada

## ABSTRACT

Martin, J. L., and D. J. Wildish. 1990. Algal blooms in the Bay of Fundy, p. 1-6. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Recent literature on harmful algal blooms indicates blooms are growing in intensity, causing more problems, and becoming more widespread throughout the world. Algal blooms have been responsible for major losses to farmed fish in western Canada, South America and Europe. Since 1978, there has been an increased use of coastal waters for salmonid aquaculture in the southwestern Bay of Fundy. A study of 17 sites was initiated in 1987 in the Fundy aquaculture region to establish baseline data of phytoplankton distribution and abundance as well as to act as an early warning indicator for the industry. Although over 150 different algal species have been observed from our samplings, those found that have caused salmonid mortalities in other areas of the world include *Alexandrium fundyense, Chaetoceros convolutus*, and *Gyrodinium aureolum*. However, all concentrations observed since our sampling began have been below the lethal levels observed when mortalities occurred in other parts of the world. During 1989, highest concentrations of *G. aureolum* (7.5 x $10^4$ cells/L) and *A. fundyense* (7.00 x $10^4$ cells/L) (as well as many other algal species) were observed offshore where the waters in the central Bay of Fundy are stratified. Inshore waters are well mixed, with organisms located throughout the water column. To date, the herring fishery is the only fishery in the Bay of Fundy that has had mortalities that have been linked to algal blooms. However, the salmonid aquaculture industry is still a relatively new industry and the threat should not be overlooked.

## RÉSUMÉ

Martin, J. L., and D. J. Wildish. 1990. Algal blooms in the Bay of Fundy, p. 1-6. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

La documentation récente sur les proliférations nuisibles d'algues indique que celles-ci deviennent de plus en plus intenses, causent davantage de problèmes et se répandent de plus en plus à travers le monde. Les proliférations d'algues sont responsables de pertes majeures dans les entreprises aquicoles de l'ouest du Canada, d'Amérique du Sud et d'Europe. Depuis 1978, on note que les eaux côtières sont davantage utilisées pour la salmoniculture dans le sud-ouest de la baie de Fundy. En 1987, on a amorcé une étude de 17 emplacements de la région aquicole de Fundy pour établir les données de base sur l'abondance et la distribution du phytoplancton et pour permettre l'établissement d'un mécanisme d'avertissement précoce pour l'industrie. Bien que plus de 150 espèces d'algues différentes aient été observées dans nos échantillons, les espèces responsables de cas de mortalités chez les salmonidés ailleurs dans le monde sont notamment *Alexandrium fundyense, Chaetoceros convolutus* et *Gyrodinium aureolum*. Cependant, toutes les concentrations observées depuis le début de notre échantillonnage sont inférieures aux concentrations létales relevées lors des mortalités signalées ailleurs dans le monde. En 1989, les concentrations les plus fortes de *G. aureolum* (7,5 x $10^4$ cellules/L) et *A. fundyense* (7,00 x $10^4$ cellules/L), comme d'ailleurs de bien d'autres espèces d'algues, ont été observées au large, là où les eaux du centre de la baie de Fundy sont stratifiées. Les eaux côtières sont bien mélangées et l'on trouve des organismes partout dans la colonne d'eau. À ce jour, les pêches de hareng sont les seules pêches de la baie de Fundy à avoir enregistré des mortalités associées à la prolifération d'algues. Cependant, la salmoniculture est une industrie encore relativement jeune et les risques ne doivent pas être négligés.

## INTRODUCTION

Algal blooms have been recognized throughout the world for thousands of years. A "bloom" occurs during a particular alga's growth period when, under the right conditions, a single cell multiplies into thousands of cells. When millions of cells are produced, a discoloration of the water can occur, whether it is red, green, brown or yellow. Regardless of color, it is called a "red tide." Blooms, however, do not have to be present in concentrations that are high enough to change the water color to cause harmful effects. In addition, high concentrations of a particular species can occur without any ill effects.

Although the majority of algal blooms do not cause any obvious problems in the environment, there are some that affect shellfish and finfish resources. The harmful blooms can be responsible for mass mortalities of different marine animals (such as shellfish, finfish and mammals) by a number of mechanisms. For example, during 1985, massive shellfish mortalities occurred off the coasts of Rhode Island and New York when *Aureococcus anophagefferens* bloomed so densely that shellfish were unable to feed (Smayda and Fofonoff 1989). Almost annually, there are also losses of farmed fish on Canada's Pacific coast from blooms of *Chaetoceros convolutus* (Eilie Stockner, pers. comm.). *C. convolutus* are bullet-shaped cells with extended spines which break off in the fish gills and penetrate membranes. Death is due either to capillary hemorrage or to suffocation from an oversecretion of mucus.

Mortalities can also be due to toxins produced by some algae. Although little is known as to how or why these algae make toxins, the toxins are among the most potent natural poisons in the world. Marine animals can accumulate lethal amounts of toxins directly during feeding or indirectly by transfer of toxins through the food chain. An example of food chain transfer may have occurred in 1987 when 14 humpback whales died in northeastern United States after eating mackerel that contained toxins produced by algae (Geraci et al., in press). A similar occurrence happened in the Bay of Fundy, eastern Canada, in 1976 and 1979. Hundreds of tons of Atlantic herring died as a result of feeding on organisms that had in turn fed on algae that produced toxins (White 1977, 1980).

Kills can occur, usually in restricted waters such as shallow bays, when a dense algal bloom dies as a result of lack of nutrients or unfavorable conditions of light, salinity or temperature. This results in bacteria growing rapidly on the mass of decaying cells, resulting in depletion of oxygen from the affected waters. Such a case occurred in the northeast part of Hong Kong in 1988 when a bloom collapsed, depleting the oxygen supply and causing mortalities of shellfish and finfish (Lam and Yip, in press).

Some algae can also affect shellfish resources with no obvious ill effects to the shellfish themselves. This is the case when marine toxins that are stored in their tissues are, upon consumption, transferred to human or other vertebrate consumers causing illness and sometimes death. The most common of the toxins are those associated with Paralytic Shellfish Poisoning (PSP) and Diarhetic Shellfish Poisining (DSP).

Harmful algal blooms seem to be becoming more widespread in frequency, magnitude and geographical extent throughout the world. More and more fish kills have been documented in recent years, including those affecting the aquaculture industry. Examples include the blooms of: *Chrysochromulina polylepis* in Norway and Sweden in 1988 (Saunders 1988; Lindahl and Dahl, in press), *Gonyaulax polygramme* in Hong Kong during 1988 (Lam and Yip, in press), and *Alexandrium*[1] *excavatum* in the Fahroe Islands in 1984 (Mortensen 1985). Before 1980, incidents where algal blooms had caused problems had been recorded from Argentina, Brazil, Canada, Chile, England, Japan, the Netherlands, Norway, New Guinea, Peru, Scotland, Spain, United States and Venezuela. Since then, Ireland, France, Sweden, Denmark, Romania, USSR, Thailand, Hong Kong, the Phillipines, India, Guatemala, Australia and New Zealand have been added to the list.

Hundreds of different species of algae bloom annually in the Bay of Fundy on Canada's Atlantic coast with no obvious effect to industry or human health. The presence of concentrations of cells high enough to discolor the water is rare to this area. However, one species known to be harmful has been in existence for a great number of years. Blooms of *Alexandrium fundyense*, an organism that produces toxins responsible for paralytic shellfish poisoning (PSP), occur annually (Gran and Braarud 1935; Martin and White 1988). Native inhabitants have historically avoided the consumption of shellfish during certain months of the year, further indicating that PSP has been evident for a number of years in this region. Shellfish accumulate toxins in their tissues while filter feeding, resulting in fatal and severe consequences to human and other vertebrate consumers. Many prime shellfish harvesting areas are closed during bloom periods each year due to unacceptable levels of toxins in shellfish.

---

[1] Formerly known as *Protogonyaulax, Gonyaulax, Alexandrium* or *Gessnerium* recently renamed to the *Alexandrium* complex.

Finfish aquaculture is a relatively new industry to Bay of Fundy waters. Most of the leases are located in the Fundy Isles region (Fig. 1) with the first established in 1978 and approximately 45 leases at present. With the increased use of inshore coastal waters for aquaculture, there is an increasing concern (worldwide) as to what effect the nutrient enrichment from food and excretion is going to have on the environment. There is also concern that introducing a large resource to an area may cause certain existing species of algae to be more noticeable or problematic.

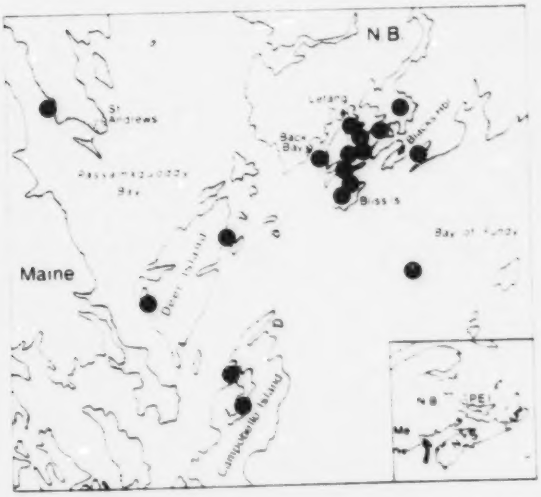


**Fig. 1. Phytoplankton sampling stations in the Fundy Isles area, New Brunswick.**

Since the aquaculture industry is relatively new to the Fundy Isles region, environmental studies were initiated in 1987 and are continuing in order to: establish some baseline data for the industry; determine whether the industry is affecting the environment; and to try to help manage the industry. Parameters being measured are: temperature, salinity, phytoplankton distribution and abundance, chlorophyll, nutrients, dissolved oxygen, suspended particulate matter and turbidity. Although each of these parameters is critical to a more comprehensive data base, this paper concentrates on a small portion of results obtained. The following are some phytoplankton results, with particular emphasis on those organisms that may cause problems to the industry.

## MATERIALS AND METHODS

Samples were collected aboard the research vessel, *Pandalus III*, from 17 sites in the Fundy Isles region (Fig. 1). Stations 2 through 15 were located within the salmonid aquaculture zone. Stations 1 and 17 were selected as indicator sites for upriver occurrences (blooms) that might affect the aquaculture sites, whereas station 16 was chosen as an offshore indicator site to act as a possible early warning of what might occur in the Bay of Fundy. Previous studies throughout the Bay of Fundy have shown that this site can give a representative indication of what is happening offshore and how cells can be transported to the inshore regions within a 1-2 wk period (Martin and White 1988; Martin, unpublished).

Sampling began in May 1987 with samples collected at the surface, 10 m, and 1 m above bottom from all sites except station 16 where samples were collected from surface, 10 m, 25 m and 50 m. Sampling was done weekly from May through October (during the major bloom periods), biweekly in April and November and monthly during the winter months - December to March. Surface samples were collected by bucket and those from depths were sampled with 1.8-L Niskin bottles equipped with reversing thermometers. Surface water temperatures were measured to ±0.1°C. Phytoplankton distribution and abundance was determined by preserving 200 mL seawater in either a 2.5% solution of formalin:acetic acid (1:1) or a 1% solution of Lugol's Iodine. After being returned to the laboratory, 50-mL subsamples were settled in Zeiss settling chambers overnight. All species greater than 5 µm were identified, including diatoms, dinoflagellates, ciliates and small zooplankton, using a Zeiss inverted microscope (eyepiece 12.5x, objectives 10-40x).

## RESULTS AND DISCUSSION

Identification of organisms revealed more than 150 different species observed in the Fundy Isles region. A complete list of organisms observed to the present can be found in Wildish et al. (1988, in prep.). There have not as yet been any finfish mortalities in aquaculture operations directly related to algal blooms in the Bay of Fundy. However, a number of organisms that have been proven to be responsible for mortalities of fish in other areas of the world have been observed in the Bay of Fundy. These are: *Gyrodinium aureolum, Chaetoceros convolutus, Alexandrium fundyense* and *Mesodinium rubrum*. These all tend to bloom annually, or nearly so. *Alexandrium* was responsible for mortalities of herring in the Bay of Fundy in 1976 and 1979. Death was as a result of paralytic shellfish toxins that had been transferred by planktonic herbivores during feeding. Marketing was not affected since the toxins concentrated in the digestive system and all fish were gutted prior to processing. Significant levels of toxins did not accumulate in the muscles. Subsequent analysis of other finfish indicated that pollock, flounder and salmon could potentially be affected (White 1981). Although cultured salmon are not as likely to accumulate the toxins through the food chain since they are fed a commercial diet, it is, however, possible for *Alexandrium* to bloom densely and be responsible for salmon mortalities. For example, concentrations reached $10^7$ cells/L, resulting in the mortality of 27 metric tonnes of salmon in 1984 in the Faroe Islands (Mortensen 1985). Although little work has been done on the mechanism of action of PSP toxins on gill tissues, the toxins seem to have affected the gill tissues and their associated functions (Mortensen 1985).

As previously mentioned, *A. fundyense* has been observed annually in Bay of Fundy waters for a number of years. Studies were conducted between 1980-84 to determine where the highest concentrations of motile cells were located during the blooms (Martin and White 1988) and where the seed beds for its overwintering form (cyst) were located during the winter and non-bloom periods (White and Lewis 1982; Martin, unpublished). Results from these studies indicated that highest concentrations of both the motile stages and the cysts were found to be in the central Bay of Fundy in the region north and east of Grand Manan. These offshore blooms of *A. fundyense* tend to be responsible for the inshore blooms that occur in Passamaquoddy Bay, the Letang and Bliss Harbour areas and other coastal areas. Figure 2 shows the results for *A. fundyense* populations (in kite diagram form) determined from our 1988 sampling at stations 16 and 3 (Fig. 1). Station 3 was representative of most of the inshore sites. During the bloom, highest numbers of cells were observed offshore at station 16, with 7.46 x $10^3$ cells/L. The highest inshore concentrations of cells were observed 1 wk later with 2.52 x $10^3$ cells/L at station 3.

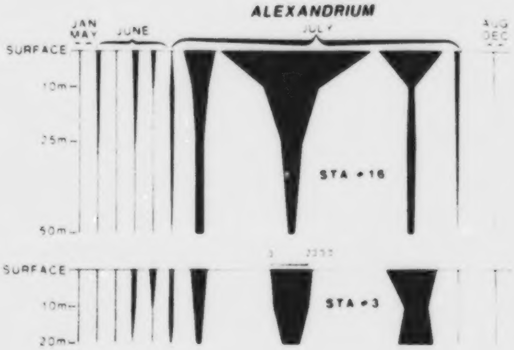


**Fig. 2.** ***Alexandrium fundyense*** **distribution at surface, 10 m, 25 m, and 50 m from station 16 and surface, 10 m, and 20 m from station 3 (Fig. 1).**

Offshore, highest concentrations were consistently observed in the surface samples. In contrast, the cells were found throughout the water column at station 3 and all other inshore sites. The motile cells offshore are able to remain in the upper stratified layers whereas our results indicate there is no stratification in the inshore waters. This is a result of tremendous inshore mixing that may be attributed to vertical mixing of the coastal waters induced by wind, tidal currents that can be greater than 100 cm/s and tidal ranges of 8 m at the mouth of the Bay of Fundy, and up to 16 m at the head (Greenberg 1983; Daborn 1986). When the *Alexandrium* bloom in the Faroe Islands occurred in 1984, the concentrations reached were approximately $10^7$ cells/L. These numbers were considerably higher than those observed from any of our sampling sites in the Fundy Isles since the sampling program began in 1987. There is only one recorded incidence to date of *Alexandrium* discoloring the Bay of

Fundy water (Martin and White 1988). This occurred in 1980 when cell numbers reached 1.8 x $10^7$ cells/L on the Nova Scotia coast of the Bay of Fundy. However, there are no aquaculture facilities located at present in this area. There are also indications that bloom densities of *Alexandrium* fluctuate on an 18.6-yr tidal cycle (caused by the change in the inclination of the moon to the equator) in the Bay of Fundy (White 1987), with the last peak in the late 1970's to 1980 (before there were many aquaculture leases). Therefore, we must presently be in a lull and the next significant rise in cell density is expected to be in the mid 1990's.

An organism, *G. aureolum*, known to have killed fish and some bottom dwellers in northern Europe, was first observed in Bay of Fundy waters in 1983 (Martin, unpublished). *G. aureolum* blooms have been observed annually since, with the exception of 1988. We have never observed more than 100 cells/mL until 1989, when there were 7.5 x $10^4$ cells/L at station 16. Mariculturists in many areas of the world are concerned about *G. aureolum* since it has been responsible for mortalities in caged salmon in areas such as Ireland (Pybus 1980), Norway (Tangen 1977) and Scotland (Roberts et al. 1983).

Although the presence of large numbers of cells can be instrumental in fish mortalities, this is not always the case. For example, in British Columbia, kills occur almost annually in aquaculture operations, and as few as 500 cells/L of the chain forming diatom, *C. convolutus*, can cause death when its spines break off and embed themselves in the gill membranes causing either capillary hemorrhage or overstimulation of mucus (Gaines and Taylor 1986). *C. convolutus* is also present in Bay of Fundy waters and has been observed around all the cage culture operations throughout the year. However, cell numbers have been generally less than 100 cells/L and the highest concentrations observed have not exceeded 500 cells/L.

Studies in the Bay of Fundy system suggest that where great tidal ranges occur, the vertical mixing can influence the whole of the water column in the shallow estuarine systems. Although nutrients are abundant and well distributed, the phytoplankton numbers tend to be low (Daborn 1986). Our studies from inshore areas where a large portion of the aquaculture industry is located tend to support this. The inshore areas tend to have fewer cells of most species than our offshore site, station 16, where stratification occurs, and which is located close to a frontal zone system where vertical mixing occurs and nutrients are also plentiful. Generally, low numbers of algal cells occur in the inshore waters in the Fundy Isles region. However, those few sites located in isolated bays or coves are at higher risk since the possibility exists that a particular algal species may bloom and become trapped and remain in the less well mixed waters. This occurred in 1977 when the ciliate, *Mesodinium rubrum*, bloomed so extensively that the water became discolored in a cove at Oven Head, New Brunswick, causing oxygen depletion in the water and resulting in major mortalities to herring trapped in a weir.

To date, there have been no salmonid mortalities that have been linked to algal blooms in the Fundy Isles region. However, the industry is still young and many different algal species are present in Bay of Fundy waters that could pose a threat. The global list of known harmful species causing mortalities to salmonids in other regions of the world is growing annually. Although the tremendous mixing and powerful tides are positive factors, the possibility is that at some time in the future the Fundy Isles aquaculture industry could face an algal problem.

## ACKNOWLEDGMENTS

We thank Francis Guptill, Aline Saulnier and Carol James for collection of samples and Murielle LeGresley for analysis of phytoplankton.

## REFERENCES

Daborn, G. R. 1986. Effects of tidal mixing on the plankton and benthos of estuarine regions of the Bay of Fundy, p. 390-413. In J. Bowman, C.M. Yentsch, and W.T. Peterson [ed.] Tidal mixing and plankton dynamics. Lecture Notes on Coastal and Estuarine Studies, 17. Springer-Verlag, Berlin and Heidelberg.

Gaines, G., and F. J. R. Taylor. 1986. A mariculturist's guide to potentially harmful marine phytoplankton of the Pacific coast of North America. Province of British Columbia Info. Rep. 10: 55 p.

Geraci, J. R., D. M. Anderson, D. G. Baden, and K. A. Steidinger (in press). Sea mammals and dinoflagellate toxins: a fatal encounter. In E. Graneli, D. M. Anderson,

L. Edler, and B. Sunström [ed.] Toxin marine phytoplankton. Proceed. Fourth Internat. Conf. Toxic Phytoplankton. Elsevier Sci. Publ. Co., Inc.

Gran, H. H., and T. Braarud. 1935. A quantitative study of the phytoplankton in the Bay of Fundy and the Gulf of Maine (including observations on hydrography, chemistry and turbidity). J. Biol. Board Can. 1: 280-467.

Greenberg, D. A. 1983. Modelling the mean barotrophic circulation in the Bay of Fundy and Gulf of Maine. J. Physicol. Oceanogr. 5: 886-904.

Lam, C. W. Y., and S. S. Y. Yip. In press. A continuous red tide incident in Hong Kong. In E. Graneli, D. M. Anderson, L. Edler, and B. Sunström [ed.] Toxic marine phytoplankton. Proceed. Fourth Internat. Conf. on Toxic Phytoplankton. Elsevier Sci. Pub. Co., Inc.

Lindahl, O., and E. Dahl. In press. On the development of the *Chrysochromulina polylepis* bloom in the Skagerrak in May-June 1988. In E. Graneli, D. M. Anderson, L. Edler, and B. Sunström [ed.] Toxic marine phytoplankton. Proceed. Fourth Internat. Conf. on Toxic Phytoplankton. Elsevier Sci. Pub. Co., Inc.

Martin, J. L., and A. W. White. 1988. Distribution and abundance of the toxic dinoflagellate *Gonyaulax excavata* in the Bay of Fundy. Can. J. Fish. Aquat. Sci. 45: 1968-1975.

Mortensen, A. M. 1985. Massive fish mortalities in the Faroe Islands caused by a *Gonyaulax excavatum* red tide. In D.M. Anderson, A.W. White, and D.G. Baden [ed.] Toxic Dinoflagellates. Elsevier Sci. Pub. Co., Inc.

Pybus, C. 1980. Observations of a *Gyrodinium aureolum* (Dinophyta) bloom off the south coast of Ireland. J. Mar. Biol. Assoc. UK 60: 661-674.

Roberts, R. J., A. M. Bullock, M. Turner, K. Jones, and P. Tett. 1983. Mortalities of *Salmo gairdneri* exposed to cultures of *Gyrodinium aureolum*. J. Mar. Biol. Assoc. UK 63: 741-743.

Saunders, R. L. 1988. Algal catastrophe in Norway. World Aquaculture 19: 11-12.

Smayda, T. J., and P. Fofonoff. 1989. An extraordinary, noxious brown-tide in Narragansett Bay. II. Inimical effects, p. 133-136. In T. Okaichi, D.M. Anderson, and T. Nemoto [ed.] Red Tides. Elsevier Sci. Pub. Co., Inc.

Tangen, K. 1977. Blooms of *Gyrodinium aureolum* (Dinophyceae) in northern European waters, accompanied by mortality in marine organisms. Sarsia 63: 123-133.

White 1977. Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as an apparent vector. J. Fish. Res. Board Can. 34: 2421-2424.

White, A. W. 1980. Recurrence of kills of Atlantic herring (*Clupea harengus harengus* caused by dinoflagellate toxins transferred through herbivorous zooplankton. Can. J. Fish. Aquat. Sci. 37: 2262-2265.

White, A. W. 1981. Sensitivity of marine fishes to toxins from the red-tide dinoflagellate *Gonyaulax excavata* and implications for fish kills. Mar. Biol. 65: 255-260.

White, A. W. 1987. Relationships of environmental factors to toxic dinoflagellate blooms in the Bay of Fundy. Rapp. P.-v. Réun. Cons. Inst. Explor. Mer 187: 38-46.

White, A. W., and C. M. Lewis. 1982. Resting cysts of the toxic red tide dinoflagellate *Gonyaulax excavata* in Bay of Fundy sediments. Can. J. Fish. Aquat. Sci. 39: 1185-1194.

Wildish, D. J., J. L. Martin, A. J. Wilson, and A. M. DeCoste. 1988. Environmental monitoring of the Bay of Fundy salmonid mariculture industry during 1986 and 1987. Can. Tech. Rep. Fish. Aquat. Sci. 1648: 44 p.

# INTERACTIONS BETWEEN ENVIRONMENT AND FISH FARMING

Arne Ervik and Pia Kupka Hansen
Division of Aquaculture
Institute of Marine Research
Bergen, Norway

## ABSTRACT

Ervik, A., and P. K. Hansen. 1990. Interactions between environment and fish farming, p. 7-10. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Studies on interaction between the environment and fish health are a field of high priority at the Institute of Marine Research. Several environmental factors are affected by fish farming, but there seems to be no simple correlation between water quality and fish health. Experiments that manipulate several environmental factors simultaneously are recommended. Effluents from fish farms normally affect only the local environment. Chemotherapeutics from medication and fish pathogenic bacteria in the sediments are considered serious environmental problems.

## RÉSUMÉ

Ervik, A., and P. K. Hansen. 1990. Interactions between environment and fish farming, p. 7-10. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'interaction entre l'environnement et la santé des poissons est un domaine de recherche à priorité élevée à l'Institute de dynamique marine. Plusieurs éléments de l'environnement sont touchés par la pisciculture, mais il ne semble pas y avoir de corrélation simple entre la qualité de l'eau et la santé des poissons. On conseille de procéder à des expériences qui portent sur plusieurs éléments de l'environnement à la fois. L'effluent des fermes aquicoles a généralement des effets sur l'environnement local seulement. Les produits utilisés en chimiothérapie et les bactéries pathogènes des poissons dans les sédiments sont considérés comme de graves problèmes environnementaux.

## INTRODUCTION

An understanding of the interaction between fish farms and the environment is fundamental for both the production of fish and the public attitude towards the new industry. The Institute of Marine Research in Bergen, Norway, which bridges research, management and industrial liaison, realized this matter early in the development of aquaculture and addressed the scientific challenges connected with the new problems. This work is being continued in the program "Interactions Environment - Fish Farming" under the Division of Aquaculture at the Institute of Marine Research.

The program covers a large field of research and various scientific disciplines. Results and progress are dependent on the collaboration between scientists from different disciplines. The Division of Aquaculture has taken the initiative in this work and has a comprehensive cooperative program within the division as well as with scientists from other institutions in Norway and abroad. Some of the central disciplines are physical and biological oceanography, diseases and genetic research, microbiology, fishery biology and the study of nutrition.

Since the research on interactions between the environment and fish farming is of a multidisciplinary nature, it is also expensive and depends on external financing. The most important supporters of the program have been the National Fisheries Research Council (NFFR), the National Pollution Control Board (SFT) and the Norwegian Fish Farms Association (NFF). A substantial part of the research conducted by the Institute of Marine Research has been a part of the national "Healthy Fish" program.

## ENVIRONMENT AND HEALTH

A good environment is necessary if an organism is to maintain health and growth. Farmed fish are no exception. It is therefore important to determine the environmental requirements of the fish and how aquaculture can be managed to satisfy those demands. When the Institute of Marine Research began its work on the environmental conditions in and around fish farms, little was known about effects of the environment on fish health. There was a lot of speculation and exaggeration about the effects of aquaculture on the surrounding sea areas. It was also believed by many that most of the diseases in fish farming were due to poor environmental conditions within the farms.

The first problem to approach was determining the actual environmental conditions at the fish farms. A number of environmental parameters were investigated between and within fish farms around the country. The results showed that the changes in the environment were less severe than expected but some the environmental parameters were dramatically affected, particularly the ammonia concentration in the net cages and the sedimentation of organic matter under the cages. The phosphate content of the seawater near the cages increased and the oxygen concentration was lower in the net cages than outside (Braaten et al. 1983; Ervik et al. 1985). Together, these changes in water quality produce an environment which is unique to fish farms and which has consequences for the health of the fish. However, under normal farming conditions, there seems to be no simple correlation between water quality and fish health.

Investigations carried out by the Institute of Marine Research to determine the effect of single parameters resulted in no clear correlation between environmental conditions and health. High ammonia concentrations might cause damage to the gills but the connection between the ammonia concentration and growth is unclear (Ervik et al. 1987). To make such observation in a realistic manner, it is probably necessary to manipulate several environmental factors simultaneously.

Realistic experiments will involve entire fish farms. The Division of Aquaculture, in collaboration with Austevoll Aquaculture Research Station and commercial farms, is currently comparing the environmental perturbations in different fish farms and resulting effects on fish health, survival and growth. Family groups are divided and placed in farms which have been chosen to produce a gradient with respect to current velocity. Health and growth of the fish are monitored, as are the environmental conditions within the farms. Close collaboration with the farmers ensures that the fish are treated identically in all the farms (Jørstad et al. 1988).

The results are still not complete but, here again, the connection between environment, fish health and growth seems to be less than direct. A good environment does not always ensure good growth or lack of diseases; the management practices on a particular farm seem crucial and may to some extent compensate for a poor environment. As expected, many families are suited to a variety of farm conditions and will grow well. Some families, however, grow well only on one or two farms, seeming to be best adapted to the specific farming operation (Jørstad et al. 1988).

## ENVIRONMENTAL IMPACT AROUND THE FISH FARMS

Norwegian fish farms are expected to produce 120,000-150,000 metric tonnes (mt) of salmon and rainbow trout in 1989. By-products of this output will be 10,000-14,000 mt of nitrogen, 1600-2000 mt of phosphate and 90,000-120,000 mt of particulate organic matter (dry weight). In addition, varying amounts of chemicals are released during the normal operation of the farm. During treatment of diseases, antibiotics or chemotherapeutics will be supplied to the environment. Responsible management of our coastal zone means understanding the impact of these substances on the life in the sea.

Understanding the effect of fish farming on the local environment began by pointing at the main problem areas. There is little measurable effect on the water within 50-100 m from a well located fish farm (Aure et al. 1988). This means that fish farming probably has little influence on the algal blooms which have affected large areas of the coast (e.g. the *Chrysochromulina* bloom in 1988). However, this does not exclude the possibility that fish farms contribute to local algal blooms in confined areas.

The sea bottom is often affected within 50-100 m of a farm. Accumulation of organic matter is often found directly beneath the net cages, with no fauna present in the sediment (Braaten et al. 1983). An increased concentration of plant nutrients is also found around the farms (Ervik et al. 1985; Aure et al. 1988). The extent to which the area is affected will depend on the location and the way the farm is run.

Investigations carried out by the Institute of Marine Research in collaboration with the University of Bergen have shown that the accumulated organic matter can contain traces of chemotherapeutics months after treatment of the fish has ceased. Furthermore, the bacteria in these sediments develop resistance towards the antibiotics (Samuelsen et al. 1988). Bacteria pathogenic to fish can survive in the sediments for months after the last appearance of disease (Husevåg et al. 1988; Husevåg and Lunestad 1989).

A model for the quantification of the environmental effect of fish farming has been developed (Aure and Stigebrandt 1989).

## PLANNING FOR THE COASTAL ZONE

No one was prepared for the rapid growth of the aquaculture industry and it was difficult to develop and enforce a long-term strategy for its expansion. By participating in the LENKA project, the Division of Aquaculture has contributed to developing guidelines for the management of the fish farming industry (Pedersen et al. 1988). This has made it possible to apply results of research quickly and directly to practical managerial regulations.

LENKA stands for "Nationwide Analysis of the Suitability of the Norwegian Coast and Watercourses for Aquaculture." It is a cooperative effort between three ministries - the Ministry of Fisheries, the Ministry of Environment and the Ministry of Local Government and Labour. The aim is, as mentioned, to formulate guidelines to be used by the authorities responsible for local development of the coastal zone. The results of the project will be printed as a report in autumn 1989.

## FURTHER WORK

An industry in progress will continually challenge research and management. It is therefore uncertain which fields of research will be most decisive for fish farming and environment in the future. In the next few years, the Division of Aquaculture will focus on the environmental effects of antibiotics, the decomposition of the particle bound organic waste and its influence on the surrounding fauna. Work on the effect of environmental factors on the health and growth of the fish will be continued.

## REFERENCES

Aure, J., A. Ervik, P. Johannesen, T. Ordemann. 1988. The environmental effects of seawater fish farms. Fisken og Havet, ISSN 071-5638 (Abstract, figure legends and table headings in English).

Aure, J., and A. Stigebrandt. 1989. Fish farming and fjords. Fiskeridirektoratets Havforskningsinstitutt, Rapp. nr. FO 8803 (in Norwegian).

Braaten, B., J. Aure, and E. Boge. 1983. Pollution problems in Norwegian fish farming. Int. Counc. Explor. Sea C.M. 1983/F:26.

Ervik, A., H. M. Elingsen, E. Farestveit, R. Fløysand, D. Ulriksen, and R. Waagbø. 1987. The effect of chronic ammonia exposure on growth, gill structure, blood chemistry and catecholamirs on Atlantic salmon (*Salmo salar*) in seawater. Int. Counc. Explor. Sea C.M. 1987/F:39.

Ervik, A., P. Johannesen, and J. Aure. 1985. Environmental effects of marine Norwegian fish farms. Int. Counc. Explor. Sea C.M. 1985/F:37.

Husevåg, B., B. T. Lunestad. 1989. Bacteria in sediment on abandoned fish farming locations in Nord Trøndelag. Internal Rep., University Bergen (in Norwegian).

Husevåg, B., B. T. Lunestad, and Ø. Enger. 1988. Bacteria in sediment from abandoned fish farm locations in Hordaland. Internal Rep., University Bergen (in Norwegian).

Jørstad, K., R. Lerøy, A. Ervik, E. Farestveit, and A. G. Eriksen. 1988. Ranking of salmon sib groups under different environmental conditions (poster and abstract). Genet. Aquacult., June 1988, Trondheim.

Pedersen, T., J. Aure, B. Bertelsen, S. Elverstad, A. Ervik, and H. Kryvi. 1988. LENKA - a nationwide analysis of the suitability of the Norwegian coast and water courses for aquaculture. A coastal zone management program. Int. Counc. Explor. Sea C.M. 1988/F:11.

Samulesen, O., V. Torsvik, P. Kupka Hansen, V. Pittman, and A. Ervik. 1988. Organic waste and antibiotics from aquaculture. Int. Counc. Explor. Sea C.M. 1988/F:14.

# SEDIMENTARY ANOXIA CAUSED BY SALMONID MARICULTURE WASTES IN THE BAY OF FUNDY AND ITS EFFECTS ON DISSOLVED OXYGEN IN SEAWATER

D. J. Wildish, V. Zitko, H. M. Akagi and A. J. Wilson
Department of Fisheries and Oceans
Scotia-Fundy Region
Biological Station
St. Andrews, New Brunswick E0G 2X0
Canada

## ABSTRACT

Wildish, D. J., V. Zitko, H. M. Akagi, and A. J. Wilson. 1990. Sedimentary anoxia caused by salmonid mariculture wastes in the Bay of Fundy and its effects on dissolved oxygen in seawater, p. 11-18. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Salmonid net-pen sites located above a depositional sediment rapidly accumulate deposits consisting of silt/clay particles, salmonid faecal matter and food wastes. This material, with its biotic community (mariculture sludge), appears to be similar to the sewage sludges of freshwater environments. The redox (Eh) values within mariculture sludges are typically negative and analysis of gases escaping from the sludge shows that they include: methane, nitrogen, carbon dioxide and hydrogen sulphide. This is consistent with anaerobic respiration processes which include some form of nitrate reduction, sulphate reduction and methanogenesis. Our studies show that the predominantly chemical oxygen demand of mariculture sludges can be a significant factor influencing the dissolved oxygen budget of salmonid net-pen sites in the Bay of Fundy.

## RÉSUMÉ

Wildish, D. J., V. Zitko, H. M. Akagi, and A. J. Wilson. 1990. Sedimentary anoxia caused by salmonid mariculture wastes in the Bay of Fundy and its effects on dissolved oxygen in seawater, p. 11-18. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Les emplacements des compartiments en filet de salmoniculture, situés sur des dépôts sédimentaires, accumulent rapidement les dépôts faits de particules de limon/glaise, de matières fécales des salmonidés et de résidus d'épuration des eaux usées que l'on retrouve en eau douce. Les valeurs d'oxydoréduction types (Eh) des boues maricoles sont négatives et l'analyse des gaz qui se dégagent des boues révèle la présence de méthane, d'azote, de dioxyde de carbone et de sulfure d'hydrogène, ce qui est conforme aux processus anaérobies (respiration) qui comportent une forme quelconque de réduction des nitrates, de réduction des sulphates et de méthanogénèse. Nos études indiquent que la demande en oxygène surtout chimique des boues maricoles peut influer d'une façon déterminante sur le bilan de l'oxygène dissous des emplacements des compartiments en filet de la salmoniculture dans la baie de Fundy.

## INTRODUCTION

The establishment of a salmonid mariculture farm significantly changes the pelagic-benthic coupling of the local area. Increased sedimentation of organic particles rich in carbon, phosphorus and nitrogen, notably salmonid faeces and waste food, may result in a rapid buildup of a mariculture sludge underneath the net-pens, particularly where erosional processes are weak and depth of seawater is limited. In such locations, an anaerobic microbial community replaces the usual aerobic one and anoxia develops within the sediment; this process has been termed souring by Gowen and Bradbury (1987). In their studies of a Scottish salmonid net-pen site in a sea loch, Brown et al. (1987) found that the impacted sediments were confined to an area of 0.6 km$^2$ under and near the net-pens. Microbial metabolism by facultative or obligate anaerobes involves a range of terminal electron acceptors other than oxygen, e.g., sulphate, carbon dioxide and fumarate (Lynch and Poole 1979; Kaspar et al. 1988) which produces highly reduced end products. The reduced compounds pass across the sediment-water interface either as gases or dissolved compounds into seawater where some of them, e.g., $H_2S$ may exert an immediate chemical oxygen demand.

All of the salmonid mariculture net-pens of the Bay of Fundy industry are sited close to shore and thus are in relatively shallow seawater (usually <20 m at mean low water (M.L.W.)). Because of this, we expected that dissolved oxygen balances at salmonid net-pen sites would be affected by sedimentary oxygen demands. This work was designed to test this hypothesis and also to determine whether or not net fouling significantly reduced the available dissolved oxygen to salmon within the net-pen.

## METHODS

Two salmonid net-pen sites within the Fundy Isles area (Fig. 1) were studied. Site 1 had ca. 8 m depth at M.L.W. and a total of 15,000 market fish on the site with 2000 fish per pen (wooden cages of 8-m diameter). During sampling in August 1988, badly fouled nets on the market fish pens were being replaced by clean ones. Site 2 had ca. 13 m depth at M.L.W. and approximately 60,000 market fish (total biomass, 210 tonnes) at the site with 2500-3500 fish per pen (22 units of plastic construction, 12 x 12 x 5 m) during sampling in August 1989. Also present were an additional 13 pens, each stocked initially with 6000 smolts (total biomass, 24 tonnes).

### Eh MEASUREMENTS

Sediments were sampled by SCUBA divers under the net-pens pushing plastic cylinders 50 cm long by 5 cm diameter into the sediment, followed by closing each end of the cylinder with a tight-fitting cap. After the core tubes were brought to the surface, the sediment could be accessed from holes drilled at 5-cm intervals in the sides covered with sticky tapes. The holes were drilled in a spiral pattern so that each could just accommodate an Orion combination redox electrode (Model 96-78). During operation, the electrode was filled with the dilute reference electrode filling solution (Orion #900001). Outputs were read on a battery-operated Orion Model 407 specific ion meter soon after the cores were landed. The mV readings obtained have been expressed relative to the normal hydrogen electrode ($E_{NHE}$) but have not been corrected for the sampling temperature observed.

### GAS SAMPLING AND ANALYSIS

Samples for gas analysis were obtained by SCUBA divers with the apparatus shown in Fig. 2. It consisted of a large inverted filter funnel and a glass bottle of 300-mL capacity which could be closed by means of pinch clamps on the rubber hoses. In operation, the divers carried the clamps in one hand and the sampler in the other. The apparatus was placed on the sediment surface until the bottle was full; this sometimes required gentle stirring of the sediment to release gas bubbles. The divers then swam to the surface holding the apparatus as shown in Fig. 2. The pinch clamps were applied just before leaving the water. Additional screw clamps were used to ensure gas tightness and the sample, kept in an ice chest, was taken to the laboratory as soon as possible (6-72 h). In the laboratory, gas samples were analyzed by gas chromatography/mass spectrometry using a Finnigan 4500 system.

### SEAWATER SAMPLING ANALYSES

Seawater samples were obtained from within or just outside a net-pen. Surface seawater was obtained in a clean bucket (0 m) and seawater at 5 m depth and 1 m off the bottom (bottom sample) in a horizontal, plastic water bottle closed by a messenger-tripped stopper at either end. Before each sample was taken, water depth was determined with a lead

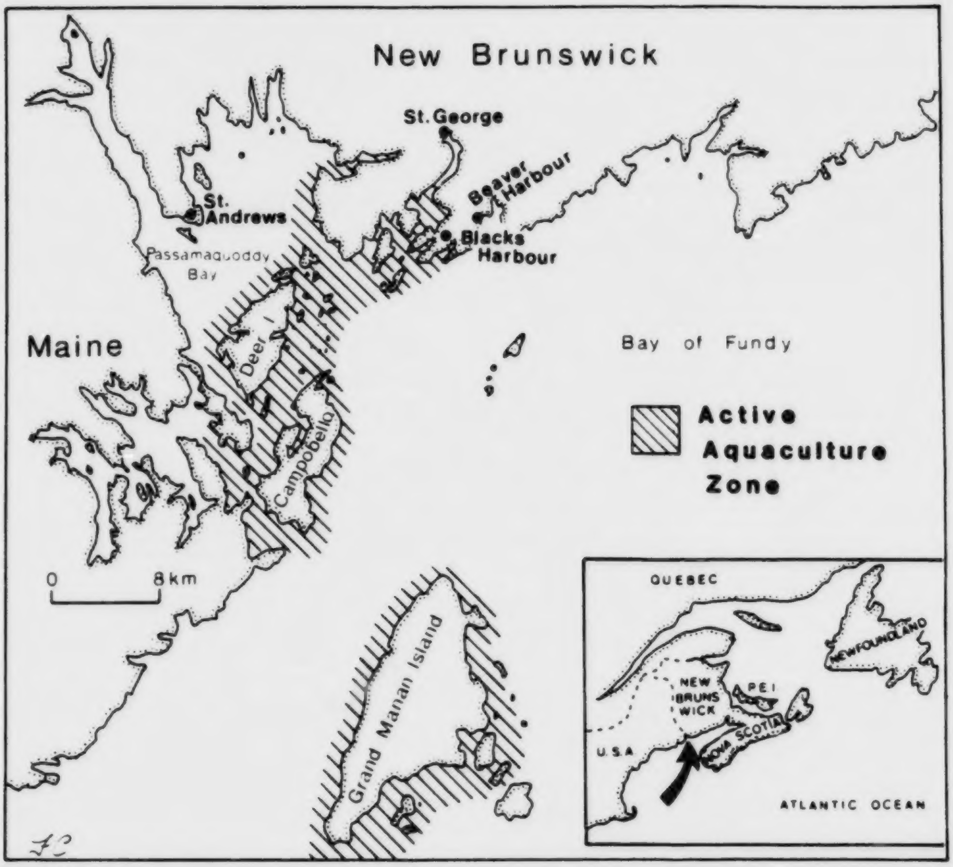

New Brunswick
St. George
Beaver Harbour
Blacks Harbour
St. Andrews
Passamaquoddy Bay
Maine
Deer I.
Campobello
Bay of Fundy
▨ Active Aquaculture Zone
0 8km
Grand Manan Island
QUEBEC
NEW BRUNSWICK
P.E.I.
NEWFOUNDLAND
NOVA SCOTIA
U.S.A.
ATLANTIC OCEAN

**Fig. 1. Map of the salmonid mariculture sites utilized by the Bay of Fundy salmon grow-out industry in 1989.**

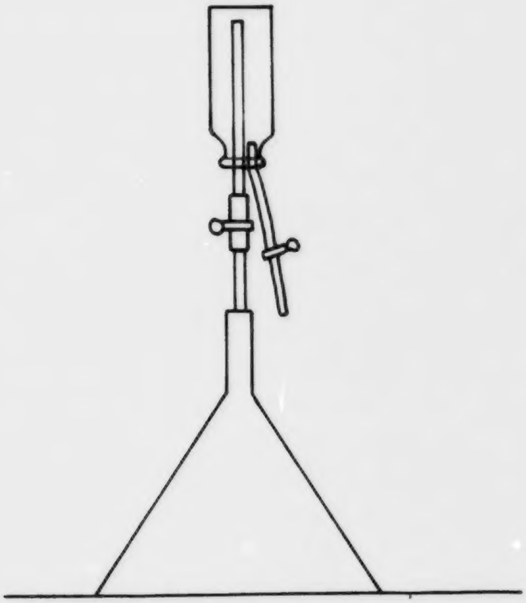


**Fig. 2. Field sampler hand-held by SCUBA divers for obtaining gas samples at the sediment-water interface.**

line marked off in 1-m steps. The capacity of the sampling bottle was 2.2 L and the contents were emptied into a clean bucket. Seawater temperature was immediately observed, to 0.1°C accuracy, with a mercury thermometer, followed by removing 300 mL for determination of dissolved oxygen by the modified Winkler method (Strickland and Parsons 1968). Manganous sulphate and alkaline iodide (1 mL each) were added in the field, followed by shaking. The samples were returned to the laboratory and titrated as soon as possible, but always within 48 h of sampling. Two hundred-mL seawater aliquots were also taken from each sample for analysis of salinity by a conductimetric method using the practical salinity (o/oo) conversion given in Anon. (1981). The dissolved oxygen value of each sample as mL/L was expressed as a percentage of the expected equilibrium dissolved oxygen at the observed temperature, salinity and sea level pressure using the equation given by Weiss (1970).

## RESULTS

### SITE 1

The sediments of the tidal cove in which the net-pens were situated were brown in color with $E_{NHE}$ = +50 to +144 mV, indicating oxic conditions. Core samples taken from directly beneath the pens showed a blackened layer for the top 25 cm of sediment with $E_{NHE}$ = -56 to -76 mV, indicating anoxic conditions. At depths greater than 25 cm in the core, the sediments were brown in color and of similar Eh values to the oxic sediment. Gas bubbles were breaking at the surface within pens containing market size fish; we have not sampled this gas.

Seawater sampling was made in two adjacent pens containing market size fish. In one of the pens, the nets had just been changed (clean net-pen) and the other in which the nets were still heavily coated with fouling organisms (fouled net-pen) common in the Bay of Fundy (see Sutterlin et al. 1981). Sampling was from 1100-1800 h on 8 September 1988 at half-hourly intervals in surface seawater only. The results (Fig. 3) show that identical minima in the percent dissolved oxygen concentration were coincident with the predicted times of low water (1700 daylight saving time, DST). The mean of 15 measurements in each pen showed that the dissolved oxygen level of seawater in the fouled net-pen was slightly less than in the clean net-pen (Table 1). The difference amounted to 1.8% of the air saturation level or 0.12 mg $O_2$/L, and a t-test (Table 1) suggests that the difference was not statistically significant.

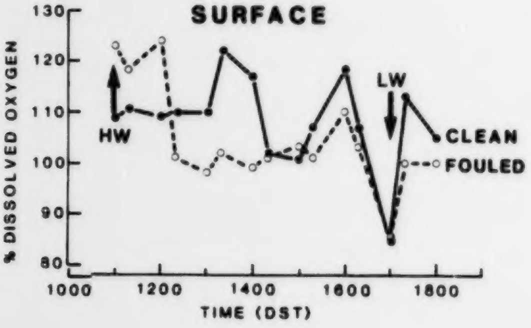


**Fig. 3. Dissolved oxygen levels as percentage of air saturation in the surface water of two net-pens at site 1 - one with clean and the other with fouled nets. Times of high (H.W.) and low (L.W.) water are indicated by arrows.**

Table 1. Dissolved oxygen as % of air saturation at site 1 between 1100 and 1800 on 8 September 1988.

| Parameter | Clean net-pen | Fouled net-pen |
|---|---|---|
| Dissolved oxygen as % air saturation | 106.4 | 104.5 |
| 1 standard error | 2.6 | 2.7 |
| N | 15 | 15 |

t = 0.734; two tail P = 0.4748.

## SITE 2

Sampling at this site was facilitated by a transect laid by SCUBA divers. Sediments were sampled at 25-ft (8-m) intervals along the transect by a hand-held corer. Most of the cores obtained contained blackened sediments and many smelled of hydrogen sulphide. Eh values measured at 5-cm intervals down the core were typically negative, indicating anoxia, and they showed a slight decline (5-20 mV) from 5-35 cm depth. The Eh sample nearest the surface (5 cm depth or less) was often markedly less negative than deeper sediments, attributable to mixing during sampling of oxic seawater and hypoxic flocculent surface organic matter. Consequently, we have used Eh values measured at 3-9 cm depth where this effect was considered to be absent. We obtained 21 cores on 4 August 1989 with $E_{NHE}$ ranging from +72 to -193 mV. The mean value for the 21 values of the transect was $E_{NHE}$ = -102 mV. Unlike site 1, there was no evidence of an oxic sediment layer underneath the mariculture sludge that we sampled.

A gas sample was also taken on 4 August 1989 at the mid point of the net-pen system where market fish were present. Gases identified included (Fig. 4) methane, nitrogen, carbon dioxide and hydrogen sulphide. Although methane appeared to be the most abundant gas, we have not been able to quantify the relative proportions of each gas. Nitric oxide was absent and the low level of hydrogen sulphide present (Fig. 4) is attributable to rapid oxidation in well buffered seawater.

Seawater sampling was begun on 15 August 1989, with hourly samples starting at 1040 and terminating at 0940 on 16 August 1989. Seawater was sampled at all three depths with the results shown in Fig. 5. A striking feature of the dissolved oxygen data is the presence of maxima and minima; five minima were present at each depth except number 4 was absent at the 5-m depth. Further analysis of the minimal dissolved oxygen values and their timing (Table 2) shows that the 0- and 5-m depth water act as a single hydrographic unit but are different from the bottom water where both concentration and timing are different.

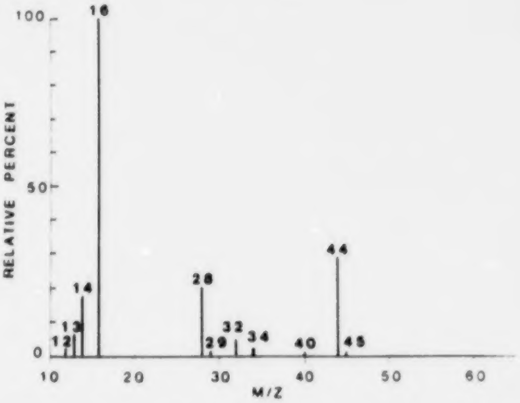


**Fig. 4. Mass spectrum of gas sample obtained from site 2 on 8 August 1989.**

## DISCUSSION

Preliminary results suggest that availability of oxygen within net-pens is affected by the degree of fouling on the nets, although statistically this is not significantly different from the clean net-pens. The two effects expected to occur in fouled net-pens are hindrance of water movement through the pen and the

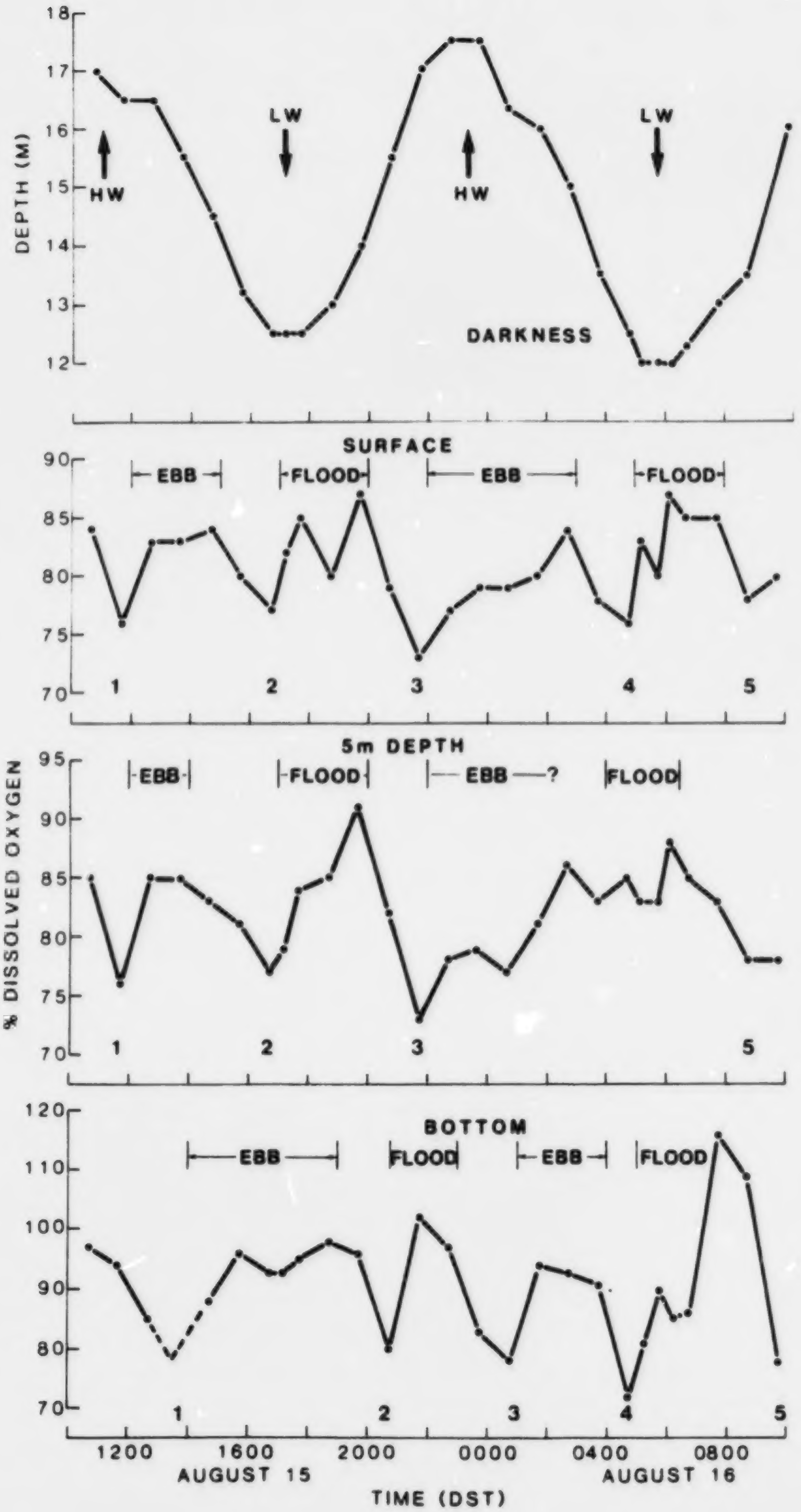


**Fig. 5.** Dissolved oxygen levels as percentage of air saturation in surface, 5 m depth and bottom water at site 2. Times of H.W. and L.W. indicated by arrows. Times of flood and ebb tidal current flows inferred from the dissolved oxygen concentration. Numbers identify the dissolved oxygen minima.

Table 2. Minimal values of dissolved oxygen and their timing during hourly sampling in the period 1040 hr, 15 August to 0940 hr, 16 August 1989.

| Minima # | Surface dissolved oxygen as % air saturation | Time DST | 5 m dissolved oxygen % air saturation | Time DST | Bottom dissolved oxygen % air saturation | Time DST |
|---|---|---|---|---|---|---|
| 1 | 76 | 1140 | 76 | 1140 | 85 | 1340 |
| 2 | 77 | 1640 | 77 | 1640 | 80 | 2040 |
| 3 | 73 | 2140 | 73 | 2140 | 78 | 0040 |
| 4 | 76 | 0440 | - | - | 72 | 0440 |
| 5 | 78 | 0840 | 78 | 0840 | 78 | 0940 |

additive biological oxygen demand of the fouling organisms themselves. As further opportunities arise to compare dissolved oxygen levels within clean and fouled net-pens, we hope to reach a conclusion as to the importance of regular net changing as far as the dissolved oxygen balance is concerned.

Eh measurements and gas sampling clearly establish that at both sites anoxic sediments are present in which an anaerobic microflora is dominant. The gases identified indicate that anaerobic metabolism involves nitrate reduction, perhaps by dissimilatory nitrate reduction as found by Kaspar et al. (1988) in sediments under a New Zealand salmonid net-pen site, by sulphate reduction and methanogenesis. Some of the reduced products exert an immediate chemical oxygen demand whose magnitude may be considerably higher than normoxic biological oxygen demands of sediments (see Poole et al. 1978). We hypothesize that the marked maxima and minima in the temporal dissolved oxygen data can be explained by alternating periods of tidal motion when dissolved oxygen saturated seawater is brought to the site, and tidal inertia when the high oxygen demands from the anoxic sediments are sufficient to begin depleting the available dissolved oxygen. In future work of this kind, we hope to measure current speeds coincident with seawater sampling to check that this explanation is feasible.

## ACKNOWLEDGMENTS

We thank J. Hunt and A. McIntyre for SCUBA diving assistance, the salmonid farmers for permission to sample their sites, Ms. Aline Saulnier and Carol James for help in analysis.

## REFERENCES

Anon. 1981. Tenth report of the Joint Panel on Oceanographic Tables and Standards. UNESCO Tech. Pap. Mar. Sci. 36: 1-25.

Brown, J. R., R. J. Gowen, and D. S. McLusky. 1987. The effect of salmon farming on the benthos of a Scottish sea loch. J. Exp. Mar. Biol. Ecol. 109: 39-52.

Gowen, R. J., and N. B. Bradbury. 1987. The ecological impact of salmonid farming in coastal waters: a review. Oceanogr. Mar. Biol. Ann. Rev. 25: 563-575.

Kaspar, H. F., G. H. Hall, and A. J. Holland. 1988. Effects of sea cage salmon farming on sediment nitrification and dissimilatory nitrate reductions. Aquaculture 70: 333-344.

Lynch, J. M., and N. J. Poole. 1979. Microbial Ecology: a Conceptual Approach. Blackwell, Oxford, 266 p.

Poole, N. J., D. J. Wildish, and D. D. Kristmanson. 1978. The effects of the pulp and paper industry on the aquatic environment. CRC Crit. Rev. Environ. Control 8: 153-195.

Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. 167: 311 p.

Sutterlin, A. M., E. B. Henderson, S. P. Merrill, R. L. Saunders, and A. A. MacKay. 1981. Salmonid rearing trials at Deer Island, New Brunswick, with some projections on economic viability. Can. Tech. Rep. Fish. Aquat. Sci. 1011: iv + 28 p.

Weiss, R. F. 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep Sea Res. 17: 721-735.

# VIBRIOSIS IN NORWEGIAN AQUACULTURE

Brit Hjeltnes and Odd Magne Rødseth
Institute of Marine Research
Bergen, Norway

## ABSTRACT

Hjeltnes, B., and O. M. Rødseth. 1990. Vibriosis in Norwegian aquaculture, p. 19-24. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Vibriosis is a disease caused by infections of *Vibrio* sp. bacteria. So far, infections by *Vibrio salmonicida* and *Vibrio anguillarum* have been the most common, costly, and widespread diseases in Norwegian aquaculture. There is evidence that *Vibrio salmonicida* plays an important role in the condition of Atlantic salmon (*Salmo salar*) known as Hitra disease. It is possible to reduce the frequency of vibriosis by vaccination.

## RÉSUMÉ

Hjeltnes, B., and O. M. Rødseth. 1990. Vibriosis in Norwegian aquaculture, p. 19-24. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

La vibriose des poissons est une maladie provoquée par la bactérie *Vibrio* sp. Jusqu'à maintenant, les infections causées par la *Vibrio salmonicida* et la *Vibrio anguillarum* ont été les maladies les plus communes, les plus coûteuses et les plus répandues de l'industrie aquicole norvégienne. Nous avons des preuves que la *Vibrio salmonicida* joue un rôle important dans une maladie du saumon de l'Atlantique (*Salmo salar*) connue sous le nom de la maladie Hitra. On peut réduire l'incidence de la vibriose par la vaccination.

## COLD-WATER VIBRIOSIS - HITRA DISEASE

Since the late 1970s, farmed Norwegian salmon (*Salmo salar*) have suffered from Hitra disease (Poppe et al. 1985). In 1986-87, the mortality reached 50-90% in several fish farms in mid- and northern Norway. Egidius et al. (1981) isolated a *Vibrio*-like bacterium from fish suffering from Hitra disease. This *Vibrio* sp. was characterized by Holm et al. (1985) and by Egidius et al. (1986) who proposed a new species, *Vibrio salmonicida*. Infection studies on rainbow trout (*Oncorhynchus mykiss*) (Egidius et al. 1981) and on Atlantic salmon (Hjeltnes et al. 1987a) showed it to be a fish pathogen capable of producing symptoms similar to Hitra disease. The infection can be transmitted from fish to fish through the water and diseased fish can be a source of infection. Fish usually become infected via the gills, but surface wounds seem to enhance the infection (Fig. 1).

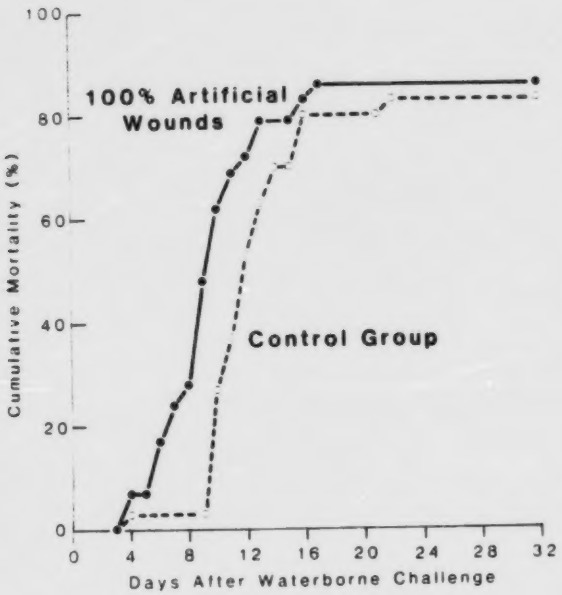


**Fig. 1. Cumulative mortality after waterborne challenge with *Vibrio salmonicida* in a group with 100% artificial wounds and in a group without wounds.**

The etiology of the Hitra disease has been discussed but part of the confusion may be due to different diseases producing the same symptoms. One of these diseases is undoubtedly a *Vibrio* infection with highest prevalence in the winter at low water temperatures, hence the name "Cold Water Vibriosis."

It has been suggested that Hitra disease arises because of a basic disorder of non-infectious origin (Fjøllstad and Heyeraas 1985; Poppe et al. 1985). The hepatic levels of copper and selenium are much lower in farmed Norwegian salmon than in wild salmon (Poppe et al. 1985). In fish suffering from the Hitra disease, the levels were further reduced. However, vitamin E and selenium supplementation in the feed of a fish population already suffering from Hitra disease did not alter the mortality rate of the fish (T. Åsgård, pers. commun.). The reduced levels of selenium in fish suffering from Hitra disease may, however, be a result of the bacterial infection. When infected with *V. salmonicida*, Atlantic salmon dying from the infection showed reduced hepatic levels of selenium, iron, and copper (Hjeltnes and Julshavn, unpubl. results; Table 1).

Because prompt therapy is essential, medical treatment of cold water vibriosis has been difficult. Since 1985, multiple resistant strains of *V. salmonicida* have been reported (Hjeltnes et al. 1987b), further emphasizing the need for a vaccine rather than an antibiotic.

In 1987, Holm and Jørgensen reported resistant fish after a double dip vaccination with a bacteria developed from a strain of *V. salmonicida*. The efficacy was recorded after a natural epizootic. Protection after natural outbreaks of cold water vibriosis has also been reported by Lillehaug (1989). Under laboratory conditions, Atlantic salmon can be effectively vaccinated against *V. salmonicida* (Hjeltnes et al. 1987c; Hjeltnes et al. 1989). Fish vaccinated twice appear to be better protected than fish vaccinated once, and the degree of protection depends on the administration route of the vaccine (Table 2, 3). The increased protection observed from booster vaccination indicates a secondary immune response in Atlantic salmon. The best protection was observed from combinations of bath and injection or dip and injection. Different ways of stimulating the immune system may be responsible for this increased protection.

Table 1. Mean concentrations of iron, copper and selenium in liver of noninfected fish and of moribund fish infected with *Vibrio salmonicida*. Standard error shown in parentheses.

| | | Basis | | | |
|---|---|---|---|---|---|
| Element | Treatment | Wet weight mg/kg | Dry weight mg/kg | Protein (mg) | Whole liver (mg) |
| Fe | Noninfected | 246 (44)* | 996 (147)* | 1370 (180)* | 514 (200) |
| | Infected | 136 (37) | 657 (156) | 854 (205) | 498 (233) |
| Cu | Noninfected | 50 (14)* | 202 (51)* | 274 (66)* | 94 (62) |
| | Infected | 20 (11) | 96 (55) | 142 (75) | 83 (65) |
| Se | Noninfected | 3.1 (0.6)* | 12.4 (2.0)* | 16.6 (2.7)* | 6.5 (2.9) |
| | Infected | 1.52(0.3) | 7.4 (1.6) | 10.4 (2.0) | 6.1 (3.2) |

*$P < 0.001$.

Table 2. Mortality in unvaccinated and vaccinated Atlantic salmon (Vaccine A) after challenge by i.p. injection of *Vibrio salmonicida* (NCMB 2262); RPP = relative percentage protection.

$$\mathrm{RPP} = \left(1 - \frac{\text{specific mortality in vaccinates \%}}{\text{specific mortality in controls \%}}\right) \times 100$$

| First vaccination | Second vaccination | No. of fish | Mortality No. | Mortality % | RPP |
|---|---|---|---|---|---|
| Unvaccinated | | 45 | 43 | 93.5 | |
| Bath | | 29 | 20 | 68.9 | 27.8 |
| Bath | Bath | 37 | 10 | 27.0 | 71.7 |
| Bath | Injection | 41 | 0 | 0 | 100 |

Table 3. Mortality in unvaccinated and vaccinated Atlantic salmon (Vaccine A) after challenge by i.p. injection of *Vibrio salmonicida* (NCMB 2262).

| First vaccination | Second vaccination | No. of fish | Mortality No. | Mortality % | RPP |
|---|---|---|---|---|---|
| Unvaccinated | | 30 | 30 | 100 | |
| Dip | | 30 | 22 | 73.3 | 26.7 |
| Dip | Dip | 30 | 8 | 26.6 | 73.4 |
| Dip | Dip | 30 | 5 | 16.6 | 83.4 |

### *VIBRIO ANGUILLARUM*

Infections due to *V. anguillarum* have been reported both in wild (Håstein and Holt 1972; Egidius et al. 1983) and farmed fish (Håstein 1975). So far, *V. ordalii* has not been isolated.

## ATLANTIC SALMON AND RAINBOW TROUT

Vibriosis caused by *V. anguillarum* has been a major disease problem in marine farming of rainbow trout, although when vaccinated, this species can be raised successfully in seawater. Atlantic salmon appear to be more resistant to *V. anguillarum* than rainbow trout. In recent years, infections with this species have become a growing problem in Norwegian salon farming. Atlantic salmon are vaccinated against vibriosis, but varied protection is claimed.

## ARCTIC CHARR

Farming of Arctic charr (*Salvelinus alpinus*) is now established in Norway. When reared in seawater or in brackish water, there is risk of exposure to *Vibrio* sp. to which Arctic charr are highly susceptible (Østhus 1976; Grotnes 1987). The gross pathology is similar to that in rainbow trout (Hjeltnes et al. 1988). Common external symptoms include haemorrhages in the head region, but petechia on the abdomen and on the operculum may also occur. Internally, the spleen is enlarged, swollen, and occasionally entirely liquified. Ascites, haemorrhages on the swimbladder and peritoneum, inflamed intestine, and extensive haemorrhages on the liver are common symptoms. Sex, maturation, and genetics may have some impact on disease resistance (Hjeltnes et al. 1988). Arctic charr can be protected against vibriosis by vaccination (Table 4), but there are indications that single vaccinations may not give long protection (Barnung and Holm 1988).

## MARINE FISH

In 1987, 30-40% of juvenile, cultured, Norwegian cod (*Gadus morhua*) died due to vibriosis. In 1988, the losses were reduced because of repeated antibiotic treatment. All the bacteria isolates so far can be classified as being the same serotype of *V. anguillarum*.

Table 4. Mortality in unvaccinated and vaccinated Arctic charr after water-borne challenge with *Vibrio anguillarum* (NCMB 2129).

| | | Mortality | | |
|---|---|---|---|---|
| | No. of fish | No. | % | RPP |
| Control | 44 | 30 | 70 | |
| Control | 35 | 22 | 62 | |
| Single vaccination (injection) | 35 | 0 | 0 | 100 |
| Single vaccination (dip) | 35 | 0 | 0 | 100 |
| Revaccinated (injection) | 35 | 0 | 0 | 100 |
| Revaccinated (dip) | 35 | 0 | 0 | 100 |

Cod can be vaccinated against vibriosis, with injection being superior to dip or bath vaccination (Fig. 2). All of these methods may be impossible to apply to extensively cultured cod. The handling can even trigger disease outbreaks as well as inducing great stress. A solution may be oral administration of the vaccine in the feed. Field studies and laboratory trials have so far been promising. The duration of protection from oral vaccination may, however, be limited.

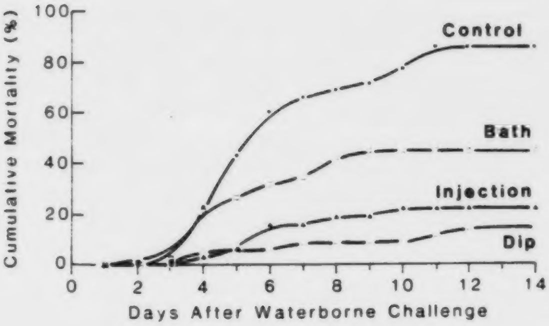


**Fig. 2. Comparison of various methods of vaccinating cod against *Vibrio anguillarum*. The fish were challenged by water-borne exposure to the bacterium 6 wk after vaccination.**

The loss of farmed juvenile turbot (*Scophthalmus maximus*) due to vibriosis were 40% in 1988. The bacteria isolated from natural outbreaks can all be classified as *V. anguillarum*. A further classification is done by lipopolysaccharide (LPS) profile and by serotyping using the system of Sørensen and Larsen (1986). Based on this information, a commercial vaccine has been produced and field trials are being carried out. This vaccine is also used in order to determine the minimum immune-competent size of the turbot.

New species in aquaculture such as Atlantic halibut (*Hippoglossus hippoglossus*) and wolffish (*Anarhichas lupus*) being with them the possibility of new strains of vibriosis. The Norwegian experience shows that vibriosis is a continuing problem in farmed fish, requiring effective medication and vaccination treatments.

## REFERENCES

Barnung, T., and J. C. Holm. 1988. Activity report 01 Jan.-30 June 1988. Arctic char rearing project. Inst. Mar. Res. Rep. No. Akva 8804 (in mimeo), 13 p.

Egidius, E., K. Andersen, E. Clausen, and J. Raa. 1981. Cold-water vibriosis or "Hitra disease" in Norwegian salmonid farming. J. Fish Dis. 4: 353-354.

Egidius, E., B. Braaten, K. Andersen, and L. Gokstad. 1983. Vibriosis in saithe (*Pollachius virens*) populations of the Norwegian coast. Rapp. P.-v. Réun. Int. Counc. Explor. Mer 182: 103-105.

Egidius, E., R. Wiik, K. Andersen, K. A. Hoff, and B. Hjeltnes. 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. Int. J. Syst. Bacteriol. 36: 518-520.

Fjøllstad, M., and A. L. Heyeraas. 1985. Muscular and myocardial degeneration in cultured Atlantic salmon (*Salmo salar* L.) suffering from 'Hitra Disease'. J. Fish Dis. 8: 367-372.

Grotnes, P. E. 1987. Bruk av villrøye i smoltproduksjon, p. 133-144. In Settefiskoppdrett inord. Norwegian Society of Aquaculture Research Symp. 3/87, ISBN 82-7445-003-5 (in Norwegian).

Hjeltnes, B., K. Andersen, H. M. Ellingsen, and E. Egidius. 1987a. Experimental studies on the pathogenicity of a *Vibrio* sp. isolated from Atlantic salmon, *Salmo salar* L., suffering from Hitra Diesease. J. Fish Dis. 10: 21-27.

Hjeltnes, B., K. Andersen, and E. Egidius. 1987b. Multiple antibiotic resistance in *Vibrio salmonicida*. Bull. Eur. Assoc. Fish. Pathol. 7: 85.

Hjeltnes, B., K. Andersen, and H. M. Ellingsen. 1987c. Vaccination against *Vibrio salmonicida*. Results from three laboratory trials. Int. Counc. Explor. Sea C.M. 1987/F:24.

Hjeltnes, B., K. Andersen, and H. M. Ellingsen. 1989. Vaccination against *Vibrio salmonicida*. The effect of different routes of administration and on revaccination. Aquaculture 83: 1-6.

Hjeltnes, B., O. M. Rødseth, and J. C. Holm. 1988. Vaccination of Arctic char (*Salvelinus alpinus*) against vibriosis. Int. Counc. Explor. Sea C.M. 1988/F:14.

Holm, K. O., and T. Jørgensen. 1987. A successful vaccination of Atlantic salmon, *Salmo salar* L., against "Hitra-disease" or coldwater vibriosis. J. Fish Dis. 10: 85-90.

Holm, K. O., E. Strøm, K. Stevsvåg, J. Raa, and T. Jørgensen. 1985. Characteristics of a *Vibrio* sp. associated with "Hitra-disease" of Atlantic salmon in Norwegian fish farms. Fish Pathol. 20: 125-129.

Håstein, T. 1975. Vibriosis in fish. A clinical, pathological and bacteriological study of the disease in Norwegian fish farms. Ph.D. Thesis, Univ. Sterling, Scotland, and National Veterinary Inst., Oslo, Norway, 196 p.

Håstein, T., and G. Holt. 1972. The occurence of *Vibrio* diseases in wild Norwegian fish. J. Fish Biol. 4: 33-37.

Lillehaug, A. 1989. Vaccination of farmed fish. Dr.Sc. Thesis, National Veterinary Inst., Oslo, Norway.

Poppe, T. T., T. Håstein, and R. Salte. 1985. Hitra Disease (Haemorrhagic Syndrome) in Norwegian salmon farming. Present status, p. 223-229. In A. E. Ellis (ed.) Fish and shellfish pathology. Academic Press, London.

Sørensen, U. B. S., and J. L. Larsen. 1986. Serotyping of *Vibrio anguillarum*. Appl. Environ. Microbiol. 51: 593-597.

Østhus, O. D. 1976. Breeding of migratory Arctic char (*Salvelinus alpinus*) in brackish and salt water. Int. Counc. Explor. Sea C.M. 1976/E:5.

# AN OVERVIEW OF THE CURRENT HEALTH STATUS OF CULTURED ATLANTIC SALMON IN THE ATLANTIC PROVINCES OF CANADA

J. W. Cornick
Fish Health Service Unit
Department of Fisheries and Oceans
Halifax Fisheries Research Laboratory
Halifax, Nova Scotia  B3J 2S7  Canada

## ABSTRACT

Cornick, J. W. 1990. An overview of the current health status of cultured Atlantic salmon in the Atlantic Provinces of Canada, p. 25-29. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The principal infectious diseases which have affected Atlantic salmon culture in the Atlantic provinces of Canada include furunculosis in New Brunswick and Newfoundland, bacterial kidney disease (BKD) in New Brunswick and Nova Scotia, vibriosis at marine sites in all four provinces, and enteric redmouth (ERM) in freshwater facilities in all provinces except Prince Edward Island, and in marine sites in Nova Scotia.

Furunculosis has been a key factor limiting the smolt supply to cages, and BKD has severely limited egg supplies to hatcheries. Vibriosis continues to affect salmon culture in marine cages but is fairly successfully controlled with vaccines and chemotherapy. ERM appears mostly in the subclinical (carrier) state, but has caused several major epizootics.

Various disease control regulations, policies and programs have been effective in limiting the spread of these diseases in the region.

## RÉSUMÉ

Cornick, J. W. 1990. An overview of the current health status of cultured Atlantic salmon in the Atlantic Provinces of Canada, p. 25-29. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Les principales maladies infectieuses touchant l'élevage du saumon de l'Atlantique observées dans les provinces maritimes canadiennes sont la furonculose au Nouveau-Brunswick et à Terre-Neuve, la maladie rénale bactérienne au Nouveau-Brunswick et en Nouvelle-Écosse, la vibriose dans les installations marines des quatre provinces ainsi que la maladie de la bouche rouge dans les installations d'eau douce de toutes les provinces sauf l'Île-du-Prince-Édouard, et dans des installations marines de la Nouvelle-Écosse.

La furonculose a eu un effet déterminant en limitant l'approvisionnement des cages en smolt et la maladie rénale bactérienne a considérablement restreint les approvisionnements en oeufs des écloseries. La vibriose continue d'avoir des effets sur la salmoniculture en cages marines, mais on réussit assez bien à la contrôler par des vaccins et par la chimiothérapie. La maladie de la bouche rouge est surtout observée à l'état subclinique (par porteurs), mais elle a causé plusieurs épizooties majeures.

Divers programmes, politiques et règlements pour la répression de la maladie ont réussi à limiter la diffusion de ces maladies dans la région.

## INTRODUCTION

Of all losses in salmon culture facilities worldwide, those related to infectious disease are probably the most drastic and certainly the most costly. Besides the value of fish lost to disease each year, vast amounts of money are spent on fish disease prevention and treatment. Although we know the impact is great, losses are difficult to quantify because hard data are seldom available. The problem is becoming more acute as market prices fall and margins of profit are reduced.

In the Atlantic provinces of Canada, the impact of disease on local salmon culture is no less important. In fact, conservative estimates have placed disease-related losses as high as 40% in some facilities. The majority of the problems are in New Brunswick (NB), where most of the commercial salmon culture activity in the region is centered. The spread of disease is most often related to extensive fish transfers between freshwater nursery sites and marine grow-out facilities. This is controlled by various regulations, policies and programs involving stock inspection and fish health certification prior to transport.

In this presentation, I discuss the principal diseases affecting Atlantic salmon culture in both freshwater and marine phases in the four Atlantic provinces as well as the effectiveness of disease control procedures currently in effect.

## PRINCIPAL DISEASES AND NUTRITION

The principal infectious bacterial diseases which have affected cultured Atlantic salmon in the Atlantic provinces of Canada are listed in Table 1, along with their distribution. In freshwater hatcheries, furunculosis and bacterial kidney disease (BKD) and, to a lesser extent, enteric redmouth disease (ERM), all affect production. In marine cages, bacterial diseases such as vibriosis, furunculosis, and BKD are the most important diseases, but ERM has caused problems in the past.

The prevalence of these diseases varies among provinces, being the highest in NB and lowest in Prince Edward Island (PEI). These differences are generally a reflection of the relative degree of stock movement associated with salmon culture in the different provinces.

Table 1. Principal diseases identified in cultured Atlantic salmon in the Atlantic Provinces of Canada[1].

| Disease | NB | NF | NS | PEI |
|---|---|---|---|---|
| Freshwater sites | | | | |
| Furunculosis | + | + | - | - |
| Bacterial kidney disease | + | - | + | - |
| Enteric redmouth | + | + | + | - |
| Marine sites | | | | |
| Vibriosis | + | + | + | + |
| Furunculosis | + | + | - | - |
| Bacterial kidney disease | + | - | + | - |
| Enteric redmouth | - | - | + | - |

[1] Based on submissions to the DFO Fish Health Service Unit Lab between 1977 and 1989.

## FURUNCULOSIS

Typical furunculosis due to *Aeromonas salmonicida var. salmonicida* has been identified in NB and Newfoundland (NF), where it is of major concern both in fresh and saltwater sites.

In NB, this disease is normally confined to freshwater sites and is endemic on several major river systems (Saint John and Restigouche) where smolts are grown. It has been identified in eight facilities where it is an important factor limiting the supply of smolts. The smolts are carefully monitored for the carrier state of the disease prior to transfer to marine cages. In spite of these controls, furunculosis reached five cage sites during the period, once in 1985 at two sites when carrier tests failed to detect infected fish, and again in 1989 at three sites when smolts at two freshwater facilities were inadvertently infected by the use of a non-sterile commercial furunculosis vaccine and subsequently moved to cages. In the first case, the disease was eradicated by the interim use of medication and subsequent stock removal. In the latest case, the disease is currently controlled by medication but, as yet, is not totally eradicated.

In 1988, furunculosis appeared for the first time in smolts in a hatchery in Bay D'Espoir, NF, and spread to marine cages with the smolts. The source of the infection is unknown. In both the hatchery and cage sites, the disease was controlled by medication and eventually eradicated by stock destruction.

## BACTERIAL KIDNEY DISEASE

Clinical BKD has been identified in Atlantic salmon in both freshwater and marine culture facilities in NB and, to a lesser extent, in Nova Scotia (NS). The disease may progress rather rapidly in fresh water but, in salt water, it tends to be slower and to persist as a chronic infection with low mortality. There is no vaccine available and the causative agent, *Renibacterium salmoninarum*, responds poorly to antibiotic treatment. Because of this, all outbreaks in fresh water have normally resulted in stock destruction. In marine cages it has, up until recently, been managed by year-class separation and careful screening of incoming smolts.

BKD is particularly troublesome because the bacteria are transmitted vertically through the egg as well as horizontally. The disease has, in the past, been an important factor limiting the availability of salmon eggs in NB. Two marine cage facilities supplying the bulk of the eggs to the aquaculture industry prior to 1987 were clinically positive for BKD, requiring careful screening of the broodstock. More clean broodstock have since been made available. Despite this fact, last year BKD was responsible for the quarantining of a major smolt facility, resulting in a shortage of approximately 0.5 million smolts. The source of the infection was traced to infected eggs which had evaded detection under the broodstock monitor program involving screening of reproductive fluids for BKD by the direct fluorescent antibody technique.

In NS, BKD is confined to Cape Breton where it exists at one hatchery and a single marine cage site. At both facilities the disease is under control and eradication attempts are under way.

## VIBRIOSIS

Vibriosis is predominantly a disease of the marine environment, where it affects salmon in all four provinces.

Both *Vibrio anguillarum* and *Vibrio ordalii* have been associated with disease outbreaks in this region. During a period from 1978-83, the disease was exclusively due to *V. anguillarum*. Between 1983 and 1985, both species were commonly isolated from epizootics. Since 1985, however, disease outbreaks have been almost exclusively due to a strain of *V. ordalii*.

The disease is generally well controlled by chemotherapy and/or vaccines. Strains commonly develop resistance to antibiotics, however, requiring periodic changes in the drugs used. Initially, oxytetracycline was the drug of choice, but recently oxolinic acid has been widely used. Available vaccines have been relatively effective against vibriosis until the recent emergence of the new *V. ordalii* strain. Fish vaccinated with early vaccines were not protected against this new strain. Recently, a new vaccine based on this strain has been introduced and it appears to be more effective.

## ENTERIC REDMOUTH

The bacterial disease ERM is most commonly found in salmon in fresh water but can be transferred to seawater in infected fish. ERM has been diagnosed in salmon in NB, NF, and NS, where it most commonly is present without any clinical symptoms (carrier state).

On occasion, however, it has caused serious epizootics in this region. In 1978, it caused a severe disease outbreak in salmon in Cape Breton, NS, resulting in the eventual destruction of a large number of fish. The source of the disease was traced to an introduction of infected trout from the U.S. midwest. The disease was eventually transmitted via the trout to Atlantic salmon in sea cages. Again in 1987, ERM was responsible for heavy losses in salmon smolts held in fresh water in an experimental aquarium at a research institution in NS. The source of the problem was linked to carrier infected fish originating from a hatchery in southwestern NS. When it occurs, this disease has responded well to oxytetracycline.

## DISEASE CONTROL PROGRAMS

A number of disease control programs operating in the region have effectively controlled the spread of these diseases (Table 2).

The National Fish Health Protection Regulations control international and interprovincial introductions of diseases through a certification and permitting system. Since 1970, no major diseases have been introduced into or between the Atlantic provinces; this is owing largely to the effectiveness of these regulations.

The maritime provinces Regional Fish Health Policy controls the movement of furunculosis, ERM, and BKD between watersheds within provinces. All lots of fish to be moved are inspected, and fish are moved only between watersheds of similar disease status. None of these diseases has been transferred by fish inspected under this program since 1983.

Specific disease control programs instituted in 1985 include a furunculosis carrier test program to prevent the movement of carrier-infected smolts to sea cages, and a broodstock monitoring program to control the vertical transmission of BKD in salmon eggs. These programs have been generally effective, although several disease outbreaks have occurred owing to breaches of the programs.

Table 2. Disease control programs in the Atlantic Provinces of Canada.

| Program | Jurisdication | Disease | Test |
|---|---|---|---|
| Fish Health Protection Regulations | International, Interprovincial | Furunculosis, ERM, BKD, IPN, IHN, VHS, *Ceratomyxa shasta, Myxosoma cerebralis* | Certification and permit |
| Regional Fish Health Policy | Interwatershed within provinces | Fununculosis, ERM, BKD | Lot inspection |
| Furunculosis Carrier Test | Fresh water to marine cage | Carrier Furunculosis | Immuno-suppression |
| Broodstock Monitor | Broodstock to hatchery | BKD | Fluorescent antibody test on reproductive fluids |

ERM - Enteric redmouth.
BKD - Bacterial kidney disease.
IPN - Infectious pancreatic necrosis.
IHN - Infectious hematopoietic necrosis.
VHS - Viral hemorrhagic septicemia.

## SUMMARY

The principal diseases affecting Atlantic salmon culture in the Atlantic provinces are furunculosis in NB and NF, BKD in NB and NS, vibriosis in all four provinces in marine cage sites, and sub-clinical ERM in all provinces except PEI.

Furunculosis has been a key factor in limiting the supply of smolts to sea cages, and BKD has severely limited egg supplies to hatcheries in the past. Vibriosis in seawater and ERM, to a lesser extent in fresh water, have caused extensive mortalities. Disease control programs as well as effective use of vaccines and chemotherapy have been effective in keeping outbreaks to a minimum.

# DETECTION OF PATHOGENS IN SUB-CLINICALLY AND CLINICALLY INFECTED SALMONIDS

Réal Lallier, Linda LeBlanc, Michel Desbiens[1] and Guy Ouellet[1]
University of Montreal
3200 Sicotte St., C.P. 5000
Saint-Hyacinthe, Québec J2S 7C6 Canada

[1]Ministère de l'agriculture, des pêcheries et de l'alimentation
96 Montée Sandy Beach, C.P. 1070, Gaspé, Québec G0C 1R0 Canada

## ABSTRACT

Lallier, R., L. LeBlanc, M. Desbiens, and G. Ouellet. 1990. Detection of pathogens in sub-clinically and clinically infected salmonids, p. 31-39. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

In this study, enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antiboty test (IFAT) were evaluated for the diagnosis of furunculosis, bacterial kidney disease and vibriosis directly from tissues of subclinically and clinically infected salmonids. Immune sera were developed in rabbits and guinea pigs; the specificity of these sera was evaluated using 29 different bacterial strains. IFAT and ELISA gave specific reactions in the homologous systems; with the 29 different bacterial strains tested, no cross-reaction was noticed. When pure cultures were used for immunofluorescence, a minimum of $10^5$ bacterial cells was necessary to obtain a positive response. With the sandwich ELISA test, only 50 *Aeromonas salmonicida* cells were necessary to produce a positive response; however, a minimum of 5 x $10^4$ *Renibacterium salmoninarum* cells were necessary to produce a positive response. IFAT or ELISA appears suitable for the diagnosis of moribund salmonids infected with *A. salmonicida* or *R. salmoninarum*. A total of 59 moribund fishes from 18 different hatcheries was analyzed; a complete correlation was obtained between ELISA and the standard methods of diagnosis. ELISA had the advantage of rapidity and facility. ELISA was compared with IFAT on 117 fishes for carrier detection. ELISA was found to be more sensitive than IFAT for the detection of fish carriers of *A. salmonicida*. However, the use of both tests increased the sensitivity. ELISA could not detect fish carriers of *R. salmoninarum* whereas IFAT could. For carrier detection, both kidney and liver should be used.

## RÉSUMÉ

Lallier, R., L. LeBlanc, M. Desbiens, and G. Ouellet. 1990. Detection of pathogens in sub-clinically and clinically infected salmonids, p. 31-39. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Dans la présente étude, la technique ELISA, soit la technique du titrage avec immuno-adsorbant lié à une enzyme, et la technique d'immunofluorescence indirecte (IFA) ont été évaluées pour le diagnostic de la furonculose, de la maladie rénale bactérienne et de la vibriose des poissons présentes directement dans les tissus de salmonidés infectés cliniquement et subcliniquement. Des immuns-sérums ont été développés chez les lapins et les cochons d'Inde; la spécificité de ces sérums a été évaluée en utilisant 29 souches bactériennes différentes. Les techniques ELISA et IFA ont produit des réactions spécifiques dans des systèmes homologues; aucune réaction croisée n'a été notée sur les 29 souches bactériennes soumises à l'essai. Lorsque l'on a utilisé des cultures pures pour la technique d'immunofluorescence, il fallait au moins $10^5$ cellules bactériennes pour obtenir une réaction positive. Dans le cas de la technique superposée ELISA, il ne fallait que 50

cellules *Aeromonas salmonicida* pour obtenir une réaction positive; toutefois il fallait au moins 5 x $10^4$ cellules *Renibacterium salmoninarum* pour obenir une réaction positive. Les techniques ELISA ou IFA semblent appropriées pour le diagnostic des salmonidés moribonds qui sont infectés par *A. salmonicida* ou *R. salmoninarum*. En tout 59 poissons moribonds provenant de 18 stations de pisciculture distinctes ont été analysés; une corrélation complète a été obtenue entre la technique ELISA et les méthodes ordinaires de diagnostic. La technique ELISA offrait l'avantage de la rapidité et de la facilité d'application. La technique ELISA a été comparée à la technique IFA sur 117 poissons pour la capacité de déceler les porteurs de maladie. La technique ELISA s'est avérée plus sensible que la technique IFA pour déceler les poissons porteurs de *A. salmonicida*. Toutefois le recours aux deux techniques accroissait l'efficacité de la détection. La technique ELISA n'était pas efficace pour déceler les poissons porteurs de *R. salmoninarum*, contrairement à la technique IFA. Pour ce qui est de la capacité de déceler les porteurs de maladie, il faudrait analyser et les reins et le foie.

## INTRODUCTION

Bacterial fish diseases limit the development of aquaculture. Furunculosis and Bacterial Kidney Disease (BKD) are two important diseases found in freshwater and seawater salmonids. Vibriosis is also an important bacterial problem but it is principally observed in seawater.

*Aeromonas salmonicida* is the causative bacterium of furunculosis. It seems that all the typical strains of *A. salmonicida* carry many common antigenic determinants (Popoff 1969; Paterson et al. 1980; McCarthy et al. 1983). In addition, some atypical strains of *A. salmonicida* also show common antigenic determinants with the typical strains (Paterson et al. 1980). BKD is caused by *Renibacterium salmoninarum*. This bacterium is antigenically homogenous and, until now, all strains isolated show similar antigenic determinants (Bullock et al. 1974; Lallier et al. 1981; Getchell et al. 1985). Two types of vibriosis are reported. One vibriosis is caused by *Vibrio anguillarum*, also identified as *V. anguillarum* biotype I, whereas the other vibriosis is caused by *Vibrio ordalii*, previously identified as *V. anguillarum* type II (Hastein and Smith 1977; Schiewe et al. 1981).

The diagnosis of furunculosis, BKD, and vibriosis is principally based on isolation of the microorganism. This procedure cannot be applied in the field. Moribund fishes have to be sent to the laboratory; frozen fishes or fishes contaminated with postmortem bacteria are not appropriate for the analysis. In addition, the culture methods require a minimum of 2 d for diagnosis of furunculosis and up to 14 d for BKD.

Recently, different laboratories have reported the possibility of using an immunological method for the detection of these diseases. Indirect immunofluorescent antibody test (IFAT) is now used to confirm standard methods for the diagnosis of furunculosis and replace standard methods for diagnosis of BKD, either Gram stain or culture (Bullock and Stuckey 1975; Paterson et al. 1979; Evelyn et al. 1981; Horiuchi et al. 1981; Lallier et al. 1981; Dutil and Lallier 1984; Lee and Gordon 1987; Smith et al. 1987). Pascho and Mulcahy (1987) developed enzyme linked immunosorbent assay (ELISA) for the detection of soluble antigens from culture of *R. salmoninarum*. This test appeared specific and no cross-reaction was noticed when using heat-extract of 11 species of bacteria. Dixon (1987) described a rapid ELISA, but before testing, the kidney has to be homogenated in buffer and centrifuged; the supernatant is used for the ELISA. In a short note, Jansson et al. (1987) reported that an immunobead assay was appropriate for the detection of *V. anguillarum* biotype I from 12 experimentally infected fishes. Smith (1981) reported that ELISA could be used for the detection of *A. salmonicida*. A dipstick-ELISA was developed for the diagnosis of furunculosis in clinically infected salmonids (Austin et al. 1986).

The main objective was to determine the specificity and sensitivity of ELISA and immunofluorescence for the detection of infected fish and carriers of *A. salmonicida* and *R. salmoninarum*.

## MATERIALS AND METHODS

### BACTERIA AND GROWTH CONDITIONS

Stock cultures were stored at -70°C until this investigation was carried out. *Aeromonas salmonicida* was grown at 18°C on FA agar (Difco) for 48 h. *Renibacterium salmoninarum* (BKD) was grown during 10 d at 18°C on charcoal agar medium (Trypsic soy agar, Difco) plus 5% bovine blood. Remaining cultures were grown for 24 h at 37°C on TSA agar. Growth was washed with a few millilitres of phosphate buffered saline (PBS) and the optical density of the suspension adjusted to 1.0 at 540 nm.

### ANTISERUM PREPARATION

Young adult rabbits and guinea pigs were used for the production of antisera against *A. salmonicida*, *R. salmoninarum*, *V. anguillarum*, and *V. ordalii*. The animals were immunized following the technique currently used in our laboratories (LeBlanc et al. 1981). Anti-*A. salmonicida* sera were absorbed with packed cells obtained from an equal volume of *V. anguillarum* suspension adjusted to DO 10.0 at 540 nm. Immunoglobulins of guinea pig sera were purified by ammonium sulfate precipitation, resuspended in PBS, dialyzed against PBS and stored at -20°C.

## INDIRECT FLUORESCENT ANTIBODY TECHNIQUE (IFAT)

IFAT described by Lee and Gordon (1987) was used. Smears of kidney and liver tissues were fixed in methanol and stained with rabbit antisera diluted 1/200. Fluorescein-labeled goat anti-rabbit globulin (Difco) and rhodamine (BBL) were used. A total of 50 fields per smear was examined.

## ELISA

Guinea pig immunoglobulins were diluted 1/50 up to 1/800 in carbonate buffer 0.1 M, pH 9.6. Double row 16-well microplates (U-16 High, Difco) were coated by filling them with 50 µL of guinea pig immunoglobulin anti-*A. salmonicida*, anti-*R. salmoninarum*, anti-*V. anguillarum* or anti-*V. ordalii* diluted 1/500. Bacterial suspensions adjusted to an optical density of 1.0 were directly used or diluted by a factor of 10 in PBS-tween buffer.

Tissue samples were mixed, using a small wooden rod, with an equal volume of PBS-tween buffer and the mixture was directly for ELISA. Bacterial suspension, 50 µL, or one Pasteur pipet drop from each tissue mixture was added to different wells. Plates were incubated at room temperature for 15 min and then washed three times with PBS-tween buffer. To each well, 50 µL of peroxydase goat anti-rabbit IgG conjugate diluted 1/400 (BioCan) were added. Plates were incubated at room temperature for 15 min and then washed three times with PBS-tween buffer. Following the addition of 100 µL of ABTS mixture (25 mL citrate buffer, 0.05 M, pH 4.0, 0.1 mL $H_2O_2$, 0.5 M, 0.25 mL of ABTS 0.004 M in $H_2O$), with further incubation at room temperature for 30 min, the optical density was recorded at 414 nm on an ELISA reader (Titertek Multiskan); the results were also read visually.

## FIELD TESTING

Moribund and healthy trout were obtained from different hatcheries. The standard method for the bacterial diagnosis of fishes was used, IFAT for BKD and culture on FA medium of the kidney for *A. salmonicida*. In addition, IFAT for ASV was done with the liver and kidney. Pieces of kidney and liver were used for ELISA. A total of 1000 Atlantic salmon of approximately 0.7 kg was transferred directly from private hatcheries into sea cages in the Baie de Gaspé. Half of these salmon received 0.7 mL of rabbit anti-*A. salmonicida* serum before the transport from the hatchery to the sea cages. Within 1 mo following the transfer into sea cages, non-vaccinated and vaccinated salmon were analyzed. Kidney and liver were used for the detection of *A. salmonicida* and *R. salmoninarum* using ELISA and immunofluorescence.

## RESULTS

Specificity of rabbit antisera was evaluated with the IFAT against the 29 bacterial strains. Doublefold dilutions, 1/50 to 1/1600, or the rabbit antisera were tested. Anti-*A. salmonicida* sera gave positive reactions with *A. salmonicida* 76-30 and 85-05 strains at a silution up to 1/800; anti-*A. salmonicida* sera diluted 1/50 gave no reaction with the other bacterial cells. Anti-*R. salmoninarum* sera diluted 1/50 gave no reaction with the other bacterial cells. Anti-*V. anguillarum* serum gave positive reactions with *V. anguillarum* and *V. ordalii* strains at a dilution of 1/800 but was negative at a dilution of 1/50 with all other bacterial cells. In the other experiments, rabbit antisa were used at a dilution of 1/400 for the immunofluorescence.

Four different ELISA tests were set up; each test consisted of guinea pig globulins, the homologous bacterium as antigen, and the corresponding rabbit antiserum. Specificity of the four ELISA tests was evaluated using the same bacterial strains used for immunofluorescence. High absorbance level, greater than 1.2, was obtained in the four homologous systems and with culture of *A. salmonicida* 85-05 in the ELISA test for the detection of *A. salmonicida*. In the heterologous systems, except in the case of *V. anguillarum* cultures in the test for the detection of *V. ordalii*, absorbances were lower than 0.2 (Table 1).

Sensitivity of the IFAT and ELISA was evaluated using different concentrations of bacteria in the homologous system. For IFAT, two drops of the bacterial suspensions were put on a microscopic slide. For ELISA, 50 µL of the bacterial suspensions were used. With IFAT, smears were positive and easy to read when 5 x $10^6$ bacterial cells were on the slide; with 5 x $10^5$ bacterial cells, it was more difficult to see fluorescent cells and results varied among observers. Table 2 shows the results obtained with ELISA. Since some responses with an absorbance of 0.28 were noticed in heterologous systems, only responses higher than 0.5 were considered positive. The ELISA tests for *A. salmonicida* could detect a minimum of 50 bacterial cells per well. The ELISA for

Table 1. Reaction of homologous and heterologous bacteria in the ELISA tests.

| Bacteria | No. of strains | ELISA tests for the detection of: | | | |
|---|---|---|---|---|---|
| | | *A. salmonicida* | *R. salmoninarum* | *V. anguillarum* | *V. ordalii* |
| *Aeromonas salmonicida* 76-30 | | 1.82[a] | 0.19 | 0.46 | 0.24 |
| *Reinbacterium salmoninarum* | | 0.16 | 1.43 | 0.16 | 0.13 |
| *Vibrio anguillarum* | | 0.20 | 0.19 | 1.47 | 0.48 |
| *Vibrio ordalii* | | 0.19 | 0.15 | 0.28 | 1.21 |
| *Aeromonas motile* | 10 | <0.12 | <0.12 | <0.12 | <0.12 |
| *Aeromonas salmonicida* 85-05 | 1 | 1.96 | 0.11 | 0.16 | <0.12 |
| *Aerococcus viridans* | 1 | <0.12 | <0.12 | <0.12 | <0.12 |
| *Bacillus* | 3 | <0.12 | <0.12 | <0.12 | <0.12 |
| *Escherichia coli* | 4 | <0.12 | <0.12 | <0.12 | <0.12 |
| *Pseudomonas* | 2 | <0.12 | <0.12 | <0.12 | <0.12 |
| *Streptococcus* | 5 | <0.12 | <0.12 | <0.12 | <0.12 |

[a] Optical density at 414 nm as determined with a Multiskan.

Table 2. Minimal number of bacteria detected in the homologous ELISA tests.

| Number of bacteria added in the well | ELISA tests for the detection of: | | | |
|---|---|---|---|---|
| | *A. salmonicida* | *R. salmoninarum* | *V. anguillarum* | *V. ordalii* |
| $5 \times 10^7$ | 1.96[a] | N.T. | 1.36 | 0.95 |
| $5 \times 10^6$ | 1.78 | 1.66 | 1.16 | 0.76 |
| $5 \times 10^5$ | 1.56 | 0.92 | 0.88 | 0.51 |
| $5 \times 10^4$ | 1.40 | 0.58 | 0.61 | 0.38 |
| $5 \times 10^3$ | 1.12 | 0.40 | 0.43 | 0.22 |
| $5 \times 10^2$ | 0.89 | 0.26 | 0.28 | 0.18 |
| 50 | 0.59 | 0.22 | N.T. | N.T. |

[a] Optical density at 414 nm as determined with a Multiskan.

the detection of *R. salmoninarum* and *V. anguillarum* could detect a minimum of 10,000 bacterial cells per well.

ELISA tests for the detection of *A. salmonicida* and *R. salmoninarum* were evaluated with trout from hatcheries with mortality problems. All fish were analyzed by ELISA and by standard diagnostic methods. All moribund fish, positive by standard methods, were strongly positive for *A. salmonicida* or *R. salmoninarum* by ELISA; no other fish tested were positive (Table 3).

Within 1 mo after their transfer into sea cages, 117 salmon were used for the research of *A. salmonicida*. As shown in Table 4, *A. salmonicida* was detected in only one vaccinated salmon. Kidney and liver of dead and apparently healthy fish were used for ELISA and IF (Table 4). Fish carriers of *A. salmonicida* and *R. salmoninarum* were detected in both groups of fish but at a different incidence; more positive fish were noticed in the non-vaccinated fish.

ELISA and IFAT were compared for the detection of *A. salmonicida* and *R. salmoninarum* in sub-clinically infected salmon. As shown in Table 5, 12 fish were positive for *A. salmonicida* by ELISA but negative by IFAT. However, three fish were positive by IFAT but negative by ELISA. For the detection of *R. salmoninarum*, 16 fish were positive by IF but no fish was positive by ELISA (Table 6).

Table 3. ELISA tests for detection of *Aeromonas salmonicida* and Renibacterium salmoninarum in fish from different hatcheries with mortality problems.

| Number of hatcheries tested | Number of fish tested | ELISA tests for *A. salmonicida* | ELISA tests for *R. salmoninarum* |
|---|---|---|---|
| Hatcheries with furunculosis 7 | 28[a] | 28[b] | 0 |
| Hatcheries with BKD 6 | 19[a] | 0 | 19 |
| Hatcheries with non-infectious diseases 2 | 4[a] | 0 | 0 |
| Hatcheries with mortalities 3 | 8[c] | 0 | 0 |

[a] Moribund fish.
[b] Number of positive fish.
[c] Healthy fish.

Table 4. Detection by ELISA and immunofluorescence of *Aeromonas salmonicida* in Atlantic salmon maintained in sea cages.

| | Number of fish analyzed | Number of fish positive for *A. salmonicida* |
|---|---|---|
| Non-vaccinated | 69 | 12 |
| Vaccinated | 48 | 1 |

Table 5. Comparison of ELISA and immunofluorescence for the detection of *Aeromonas salmonicida* in salmon carriers.

| Immunofluorescence | ELISA<br>Positive | <br>Negative | <br>Total |
|---|---|---|---|
| Positive | 0 | 3 | 3 |
| Negative | 12 | 213 | 225 |
| Total | 12 | 216 | 228 |

Table 6. Comparison of ELISA and immunofluorescence for the detection of *Renibacterium salmoninarum* in salmon carriers.

| Immunofluorescence | ELISA<br>Positive | <br>Negative | <br>Total |
|---|---|---|---|
| Positive | 0 | 16 | 16 |
| Negative | 0 | 212 | 212 |
| Total | 0 | 228 | 228 |

## DISCUSSION

The detection of *A. salmonicida, R. salmoninarum*, and *V. anguillarum* were compared in this study using ELISA and IFAT. The same rabbit antisera were used for ELISA and IFAT. The specificity of these antisera was first evaluated with IFAT. These antisera appeared specific; no cross-reaction was observed at a dilution of 1/50, whereas homologous reactions still occurred at a dilution of 1/800.

We found that, using purified guinea pig IgG instead of whole serum, the sensitivity increased and the background level decreased in the ELISA (data not shown). A complete checker board titration was done in order to determine the optimal dilutions of rabbit and guinea pig antisera. The optimal dilution of teh conjugate appears to vary from lot to the other or from producer to producer. For each new lot of conjugate, the optimal diltion has to be determined. The incubation time of the antiserum was also evaluated. Incubation for a longer period did not increase the sensitivity, but the intensity of the color was higher in both positive and negative controls.

Pure cultures of *A. salmonicida* and *R. salmoninarum* were used to compare sensitivity of ELISA and IFAT. With the aid of the Multiskan, ELISA seemed more sensitive than the IFAT for the detection of both *A. salmonicida* and *R. salmoninarum.* It was very difficult to see fluorescent bacterial cells on a smear containing fewer than $10^5$ cells, whereas at this concentration of bacteria an optical density higher than 0.9 was obtained.

Moribund fish from naturally occurring diseases were analyzed. A total of 59 moribund fish from 18 different hatcheries were analyzed by ELISA and the standard diagnosis method. No false positive or false negative results were noticed. Infected fish gave an optical density higher than 0.7, whereas with non-infected fish, the response was always lower than 0.3. Results were also read without the aid of the Multiskan; the difference in the color intensity between the infected and non-infected fish was noticeable, and the interpretation could be done without any difficulty. ELISA with kidney from BKD clinically infected fish gave an optical density at A450 nm higher than 1.0 and was visually positive. In two cases, kidney from moribund fish without macroscopic signs gave an optical density at A450 nm higher than 1.0 in the ELISA; the final diagnosis of BKD was further confirmed by the standard procedure of histopathology.

Treatment with rabbit anti-*A. salmonicida* of experimentally infected fish with *A. salmonicida* appears to be very effective (Marquis and Lallier 1989). In this study, we observed that A. salmonicida was detected in 17% of non-treated salmon, whereas only 2% of salmon treated with rabbit anti-*A. salmonicida* were carriers of *A. salmonicida.* These results could suggest a beneficial effect of the treatment with rabbit antiserum on the reduction of fish carriers of *A. salmonicida.*

ELISA enables the detection of bacteria directly in the fish tissues without any treatment of the tissues. When naturally clinically-infected fish were used, a complete visual correlation was noticed between the final diagnosis and ELISA. ELISA can detect *A. salmonicida, R. salmoninarum,* or *V. anguillarum* from a piece of tissue, without any treatment, in less than 2 h. With sub-clinically infected fish, whereas ELISA seems appropriate for the detection of *A. salmonicida*, only IFAT gave positive results for the detection of *R. salmoninarum* at the carrier level.

## ACKNOWLEDGMENTS

This work was supported by the "Ministère de l'éducation - FCAR actions spontanées" and by the "Ministère de l'alimentation et des pêcheries du Québec." We gratefully acknowledge Miss Francine Bernard for the technical assistance in the ELISA.

## REFERENCES

Austin, B., I. Bishop, C. Gray, B. Watt, and J. Dawes. 1986. Monoclonal antibody-based enzyme-linked immunosorbent assays for the rapid diagnosis of clinical cases of enteric redmouth and furunculosis in fish farm. J. Fish Dis. 9: 469-474.

Bullock, G. L., and H. M. Stuckey. 1975. Fluorescent antibody identification and detection of *Corynebacterium* causing kidney disease of salmonids. J. Fish. Res. Board Can. 32: 2224-2227.

Bullock, G. L., H. M. Stuckey, and P. K. Chen. 1974. Corynebacterial kidney disease of salmonids: growth and serological studies on the causative bacterium. Appl. Environ. Microbiol. 28: 81-814.

Dixon, P. F. 1987. Detection of *Renibacterium salmoninarum* by enzyme-linked immunosorbent assay (ELISA). J. Appl. Ichthyol. 3: 77-82.

Dutil, J.-D., and R. Lallier. 1984. Testing bacterial infection as a factor involved in the mortality of catadromous eels (*Anguilla rostrata*) migrating down the St. Laurent estuary (Canada). Naturaliste can. (Rev. Ecol. Syst.) 111: 395-400.

Evelyn, T. P. T., J. E. Ketcheson, and L. Prosperiporta. 1981. The clinical significance of immunofluorescence-based diagnoses of the bacterial kidney disease carrier. Fish Pathol. 15: 293-300.

Getchell, R. G., J. S. Rohovec, and J. L. Fryer. 1985. Comparison of *Renibacterium salmoninarum* isolates by antigenic analysis. Fish Pathol. 20: 149-159.

Hastein, T., and J.-E. Smith. 1977. A study of *Vibrio anguillarum* from farm and wild fish using principal components analysis. J. Fish Biol. 11: 69-75.

Horiuchi, M., T. Sato, H. Takagi, and K. Totsuka. 1981. Preliminary examinations on application of indirect immunofluorescence to diagnosis of five analogous bacterial diseases of cultured eels. Bull. Jap. Soc. Fish. 47: 561.

Jansson, E., B. Sundqvist, E. Rivera, A. Thuvander, and O. Ljungberg. 1987. A solid phase immunoassay for the rapid detection of *Vibrio anguillarum* in infected rainbow trout. Aquaculture 67: 206.

Lallier, R., G. Olivier, P. Chartier, C. Turcotte, B. Dion, and P. Paulhus. 1981. Présence de la maladie bactérienne du rein chez les salmonidés du Québec. J. Vét. Can. 22: 226-229.

LeBlanc, D., K. R. Mittal, G. Olivier, and R. Lallier. 1981. Serogrouping of motile *Aeromonas* species isolated from healthy and moribund fish. Appl. Environ. Microbiol. 42: 56-60.

Lee, E. G.-H., and M. R. Gordon. 1987. Immunofluorescence screening of *Renibacterium salmoninarum* in the tissues and eggs of farmed chinook salmon spawners. Aquaculture 65: 7-14.

Marquis, H., and R. Lallier. 1989. Protection of brook trout (*Salvelinus fontinalis*) against *Aeromonas salmonicida*, by passive immunisation after a bath challenge. J. Fish Dis. 12: 233-240.

McCarthy, D. H., D. F. Amed, K. A. Johnson, and J. V. Bloom. 1983. *Aeromonas salmonicida* determination of an antigen associated with protective immunity and evaluation of an experimental bacteria. J. Fish Dis. 6: 155-174.

Pascho, R. J., and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. Can. J. Fish. Aquat. Sci. 44: 183-191.

Paterson, W. D., D. Douey, and D. Desautels. 1980. Relationship between selected strains of typical and atypical *Aeromonas salmonicida, Aeromonas hydrophila* and *Haemophilus piscium*. Can. J. Microbiol. 26: 588-598.

Paterson, W. D., C. Gallant, D. Desautels, and L. Marshall. 1979. Detection of bacterial kidney disease in wild salmonids in the Margaree River system and adjacent waters using indirect immunofluorescent antibody technique. J. Fish. Res. Board Can. 36: 1464-1468.

Popoff, M. 1969. Etude sur les *Aeromonas salmonicida*. Caractéristiques biochimiques et antigéniques. Rech. Vét. 3: 49-57.

Schiewe, M.-H., T.-J. Trust, and J.-H. Crosa. 1981. *Vibrio ordalii* sp nov.: a causative agent of vibriosis in fish. Curr. Microbiol. 6: 343-348.

Smith, A. M., O. L. Goldring, and G. Dear. 1987. The production and methods of use of polyclonal antisera to the pathogenic organisms *Aeromonas salmonicida, Yersenia ruckeri* and *Renibacterium salmoninarum*. J. Fish Biol. 31: 225-226.

Smith, D. D. 1981. Enzyme-linked immunosorbent assay (ELISA) for the detection of *Aeromonas salmonicida* in diseased fish tissue. Develop. Biol. Stand. 49: 97-100.

# GENETIC STUDIES ON FARMED FISH: GENOTYPE x ENVIRONMENT INTERACTION AND GENETIC EFFECTS ON NATIVE GENE POOLS

Knut E. Jørstad
Institute of Marine Research
Division of Aquaculture
N-5024 Nordnes-Bergen, Norway

## ABSTRACT

Jørstad, K. E. 1990. Genetic studies on farmed fish: genotype x environment interaction and genetic effects on native gene pools, p. 41-47. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Population genetic studies of fish have been carried out at the Institute of Marine Research in Bergen for more than 25 yr. The work started with the application of electrophoretic methods in stock identification studies of commercially important fish stocks. In the 1970s, genetic studies in connection with salmonid farming became important. More recently, several experiments focusing on genotype x environment interactions have been carried out. Some preliminary results of growth of salmon sib groups under different environmental conditions are presented in this contribution. Recently, much attention has been focused on the potentially harmful genetic effects on wild populations due to large-scale release programs and/or escaped farmed fish. The development of gene markers in fish and the application of genetically tagged stocks in controlled interaction studies are discussed.

## RÉSUMÉ

Jørstad, K. E. 1990. Genetic studies on farmed fish: genotype x environment interaction and genetic effects on native gene pools, p. 41-47. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'Institut de recherche marine de Bergen étudie la génétique des populations de poisson depuis plus de 25 ans. Le travail a commencé avec l'application de méthodes d'analyse électrophorétique dans les études sur l'identification des divers stocks de poissons à valeur commerciale importante. Dans les années 1970, les études génétiques sont devenues importantes pour les salmoniculteurs. Plus récemment, plusieurs expériences ont tenté de déterminer les interactions entre le génotype et le milieu. Le présent document donne les résultats préliminaires d'une étude sur la croissance de groupes de saumon de la même famille qui sont élevés dans des conditions écologiques différentes. Récemment, on a beaucoup parlé des effets génétiques nuisibles que les programmes étendus de remise à l'eau et la fuite des poissons d'élevage peuvent avoir sur les populations sauvages. Le document traite aussi de l'élaboration de marques génétiques pour le poisson et de l'utilisation de stocks de poisson marqués génétiquement dans des études contrôlées sur les interactions.

## INTRODUCTION

Genetic variation is usually described as individual variation within a group or population and as a collection of genetically differentiated populations within a species. The genetic material utilized in aquaculture is initially based on the genetic resources found in wild populations (Shaklee 1983). A simple flow diagram of the relationships between cultured organisms and wild populations is presented in Fig. 1.

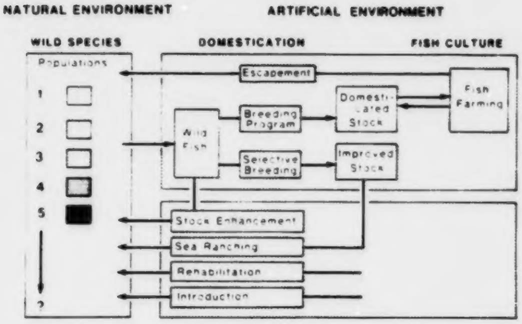


**Fig. 1. Flow diagram illustrating different lines of interaction between farmed and wild populations.**

The diagram illustrates the most important areas of interest with regard to population genetic studies carried out in Bergen during the last decade. These include: 1) stock identification studies of commercially valuable fish species by means of biochemical methods; 2) domestication and breeding in connection with salmonid farming; and 3) genetic studies incorporated in large-scale release programs of fish. In the last mentioned area, genetic effects on wild populations due to escapement of farmed fish are included.

In this contribution, however, preliminary results from two kinds of investigation recently performed are described. The first study deals with genotype x environment interaction under farming conditions. The second one examines the potential genetic interaction between farmed fish and wild stocks and suggests that genetically marked strains should be used in investigations of controlled escapement.

## BREEDING PROGRAMS AND GENOTYPE X ENVIRONMENT INTERACTION

The large-scale salmon breeding program in Norway is aimed at developing a single, high performance strain for the salmon industry. Commercially important traits such as fast growth, late maturity, meat quality, and disease resistance are considered to be the main characters and form the basis for development of a farmed strain.

The salmon farms are distributed in fjord and coastal areas which differ in environmental conditions. The investigation was initiated in 1986 when about 3000 1-yr smolts from 70 different full sib groups were placed in four commercial fish farms. The fish were tagged by finclipping and cold branding and could be identified to family group. The environmental conditions differed in a number of ways, especially the degree of water exchange which was assumed to be the most important one. In 1987, a new collection of salmon sib groups were placed in three of the same farms.

Environmental parameters such as temperature, ammonia, and oxygen concentrations were measured routinely (Jørstad et al. 1988). Feed consumption, mortalities, and mean growth were recorded at regular intervals. The fish populations were measured after about 1 yr in the sea and identified to family group. Due to technical reasons and handling problems at some of the farms, up to 15% of the fish were not identifiable to family group. Production results were obtained at harvest 1 yr later, and as all measurements were carried out on dead fish, the fraction of nonidentified fish was reduced to 3-5%. However, only data from properly characterized fish are presented in the tables.

The production results obtained at each farm for the two year-classes are shown in Tables 1 and 2. For the 1985 year-class, one farm (B) had substantial ice problems during the first winter; this reduced the number of fish in the pen as well as growth of the fish. This farm was characterized by very low water exchange and unsuitable environmental conditions (Jørstad et al. 1988). Later, the farm was moved to a better locality and, for this reason, measurements of the 1986 year-class on this farm were not carried out (see Table 2).

Table 1. Production results of the 1985 year-class after 15 and 22 mo in seawater. At harvest, all the fish were measured and 95% of the individuals were identified to family group.

| | Salmon farms | | | |
|---|---|---|---|---|
| | A | B | C | D |
| No. fish | | | | |
| 14 mo | 2359 | 1435 | 2942 | 3522 |
| 23 mo | 2386 | 1351 | 2987 | 3200 |
| No. families | | | | |
| 14 mo | 76 | 70 | 79 | 28 |
| 23 mo | 79 | 79 | 80 | 78 |
| Families >10 fish | | | | |
| 14 mo | 66 | 50 | 67 | 69 |
| 23 mo | 69 | 52 | 69 | 68 |
| Mean length (cm) | | | | |
| 14 mo | 43.3 | 47.7 | 51.0 | 49.4 |
| 23 mo | 62.3 | 64.0 | 66.6 | 61.2 |
| Mean weight (kg) | | | | |
| 14 mo | 1.4 | 1.2 | 1.5 | 1.4 |
| 23 mo | 2.9 | 2.9 | 3.4 | 2.7 |

Table 2. Production results at different farms after 15 and 22 mo in seawater. At slaughtering, all fish of the 1986 year-class were measured and 95% were identified to family group.

| | Salmon farms | | |
|---|---|---|---|
| | A | C | D |
| No. fish | | | |
| 15 mo | 4084 | 4608 | 4910 |
| 22 mo | 3505 | 3857 | 4409 |
| No. families | | | |
| 15 mo | 69 | 69 | 67 |
| 22 mo | 67 | 67 | 68 |
| Families >10 fish | | | |
| 15 mo | 61 | 61 | 60 |
| 22 mo | 59 | 60 | 61 |
| Mean length (cm) | | | |
| 15 mo | 54.0 | 53.8 | 53.6 |
| 22 mo | 61.9 | 62.3 | 64.6 |
| Mean weight (kg) | | | |
| 15 mo | 1.8 | 1.9 | 1.8 |
| 22 mo | 2.6 | 2.7 | 3.2 |

The production results expressed as mean weights, including all fish measured, varied substantially between farms. For the first year-class (Table 1), the difference between the highest and lowest mean weight was 0.7 kg. For the last year-class, the difference was 0.6 kg. Obviously, such differences between farms using the same genetic material have important economic implications. For the 1986 year-class, when the three different farms received approximately the same number of smolts, the difference between farm A and D at harvest constitutes nearly 5 tonnes of salmon. The same salmon sib groups were also kept at the Akvaculture Station Austevoll (data not shown). The mean weight obtained during the same period was as high as 3.9 kg.

Based on family tagging, the mean weights of each family were estimated for all farms at harvest. Only families with more than 10 individuals were used in the comparisons. As demonstrated for farm D which has been used as a reference (Fig. 2, 3), the 10 best ranking families seemed to be superior compared to the other families. The same observations were seen for all farms investigated, but in those cases, more than 30% of the 10 best ranking families were different compared with the ranking at farm D. A rank correlation test (Kendall's test of concordance, including significance test, SPSS/PC statistical computer program package) revealed significant differences among the 10 ranking families in the 1985 year-class (W = 0.387; chi-square = 11.62; df = 3; P = 0.009) as well as for the 1986 year-class (W = 0.41; chi-square = 8.21; df = 3; P = 0.017). Significant differences in the ranking were also detected in all pair-wise comparisons between the farms.

Direct comparisons of mean weights of each family of the 1985 year-clsss at different farms are summarized in Fig. 2. As shown, farms A and D have a similar ranking of families. With reference to breeding programs, the ranks of the 25 best families were compared and no significant differences were detected between farms A and D. Some high ranking families at farm A are, however, found in the lower part among the low ranking families (such as no. 33 and 38) at farm D. This phenomenon is even more clearly expressed in the comparisons with farms B and C. At both of these farms, superior families were found

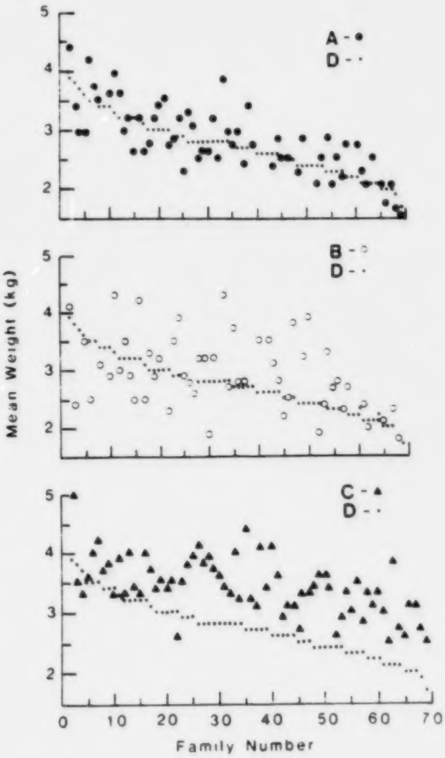


**Fig. 2. Comparisons of mean weights of the 1985 year-class salmon sib groups measured at different farms. The ranking profile obtained at farm D is used as a reference.**

**Pair-wise comparison of the ranking (Kendall's test, SPSS/PC package) of the 25 best families:**

**Farm A-D: W = 0.0076; Chi-square = 0.18;**
**P = 0.66**
**Farm B-D: W = 0.19; Chi-square = 4.84; P = 0.028**
**Farm C-D: W = 0.24; Chi-square = 6.0; P = 0.014**

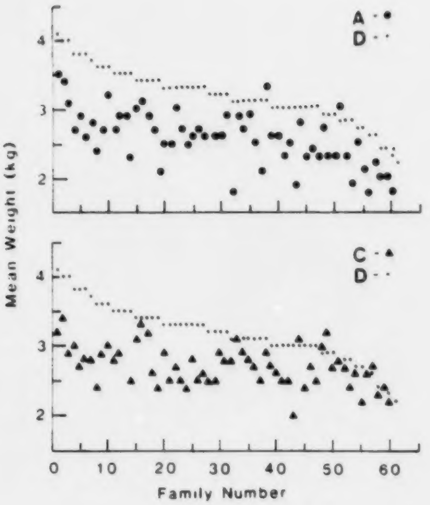


**Fig. 3. Comparisons of mean weights of the 1986 year-class of salmon sib groups measured at different farms. The ranking profile obtained at farm D is used as a reference:**

**Pair-wise comparisons of ranking of the 20 best families:**

**Farm A-D: W = 0.16; Chi-square = 3.20; P = 0.074**
**Farm C-D: W = 0.16; Chi-square = 3.20; P = 0.074**

which had a low ranking number at the reference farm. The largest differences were detected between farm C and the reference farm. Pair-wise comparison of the ranks of the 25 best families demonstrated significant differences for both farms (see legend to Fig. 2).

A similar picture was obtained for the second year-class and the data are presented in Fig. 3. In this case, both farms A and C had superior families which were found in the lower part of the ranking families at the reference farm. In comparison of the ranking of the 20 best families, the differences between the farms were only close to significance (see legend to Fig. 3).

The data presented are the measurements of mean weights at harvest of the same families at farms under different environmental conditions. The variation in mean production results and mean weights of the individual families observed between farms could be due to variation in physical environmental conditions, feeding regime, and health status. Such information is available, and at present more comprehensive data processing and statistical treatment are in progress.

## A VISUAL METHOD TO EXAMINE THE GENETIC INFLUENCE OF RELEASED FISH ON NATIVE GENE POOLS

Breeding programs related to fish farming are aimed at improving the strain performance under farming conditions. Successful artificial selection for economically important traits result in domesticated strains which are genetically different from wild populations. As demonstrated in Fig. 1, a breeding program is initially based on wild fish caught from natural stocks. A number of studies have dealt with genetic changes during the domestication process (for review, see Skaala et al. 1990). Intensive cultivation of an organism usually implies alterations of the fish's ability to perform under natural conditions. Thus, escapement of farmed fish and introgression with the wild stocks are believed to be a genetic threat to the integrity of locally adapted wild stocks.

During the last 10 yr, the salmon production in Norway has increased to over 100,000 tonnes annually, and ascape of farmed fish has been substantial during recent years. Significant numbers of mature salmon, characterized by deformities of fins, opercula, and snout, have been detected during salmon spawning runs in Norwegian rivers (Directorate for Nature Management 1989). Very little is known, however, about the spawning and reproductive success of the escapees and the fitness of their offspring.

The genetic influence on native gene pools from escaped farmed fish could be studied by using biochemical markers like those applied for evaluation of stocking programs (Allendorf and Utter 1979; Chilcote et al. 1986; Taggart and Ferguson 1984, 1986). With respect to the present problem connected with escaped salmon in Norway, no genetically marked strain is available. Fine spotted brown trout (*Salmo trutta* L.) have been described recently by Skaala and Jørstad (1987). The spotting character has been shown to be inherited (Skaala and Jørstad 1988). The fine spotted individuals are characterized by small, dense spotting on the main body and some larger spots are found in the eyes. The fine spotted trait is shown in Fig. 4 for adults and juveniles. Controlled crossing experiments demonstrate that different offspring groups could be identified at the fry stage. In addition, the fine spotted

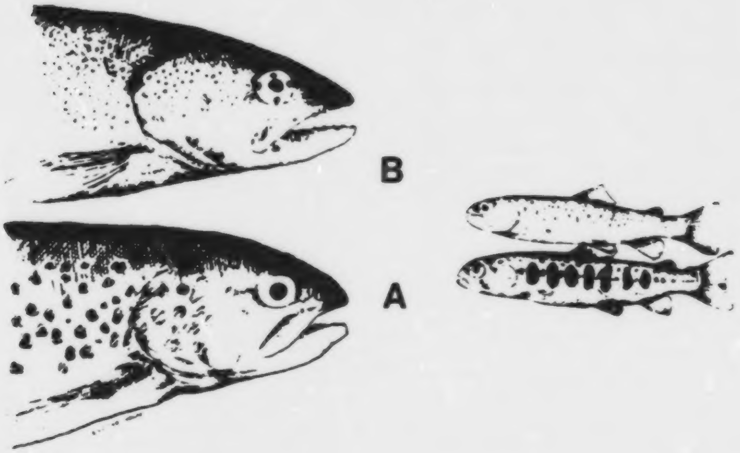


**Fig. 4. Diagram comparing normal and fine spotted variants of brown trout (*Salmo trutta* L.). A: normal spotting pattern; B: fine spotted pattern (modified after Skaala and Jørstad 1987; Skaala and Jørstad 1988).**

population is also nearly fixed for a rare allele at an allozyme locus.

A broodstock of fish with the genetic properties mentioned has now been established and is going to be used in field studies. Genetic and biological studies of wild trout in selected localities have already been started and we will use the fine spotted trout as a model population to investigate gene interaction between local stocks and released/escaped fish. By releasing mature fine spotted individuals of both sexes, a gene pulse experiment is thereby started. The reproductive success of farmed fish, introgression, and survival of the offspring could be investigated and the genetic influence on the native population can be monitored in the following generations.

## ACKNOWLEDGMENT

The study of salmon families under different environmental conditions is a joint project with Arne Ervik which also includes Rita Lerøy, Eva Farestveit, and Anne Grete Eriksen. The work on fine spotted brown trout has been carried out mainly by Øystein Skaala at this Department. I also appreciate the comments and suggestions of J. Bailey, G. Friars, and the editor on the first version of the manuscript.

## REFERENCES

Allendorf, F., and F. Utter. 1979. Population genetics, p. 407-454. In W. S. Hoar, D. J. Randall and J. R. Brett (ed.) Fish Physiology, Vol. VIII. Academic Press, London.

Chilcote, M. W., S. A. Leider, and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans. Am. Fish. Soc. 115: 726-735.

Directorate for Nature Management. 1989. Rørnt oppdrettsfisk i vassdrag 1988. Rapport nr. 3 - 1989 (in Norwegian).

Jørstad, K. E., R. Lerøy, A. Ervik, E. Farestveit, and A. G. Eriksen. 1988. Ranking of salmon sib groups under different environmental conditions, p. 322. In T. Gjedrem (ed.) Genetics in aquaculture. Aquaculture 85.

Shaklee, J. B. 1983. The utilization of isozymes as gene markers in fisheries management and conservation, p. 213-247. In M. Ratazzi, J. G. Schandalios and G. S. Whitt (ed.) Isozymes: current topics in biological and medical research, Vol. 11: Medical and other applications. Alan R. Liss, Inc., NY.

Skaala, Ø., G. Dahle, K. E. Jørstad, and G. Naevdal. 1990. Interactions between natural and farmed fish populations: information from genetic markers. J. Fish Biol. 36: 449-460.

Skaala, Ø., and K. E. Jørstad. 1987. Fine-spotted brown trout, its phenotypic description and biochemical genetic variation. Can. J. Fish. Aquat. Sci. 44: 1775-1779.

Skaala, Ø., and K. E. Jørstad. 1988. Inheritance of the fine spotted pigmentation pattern of brown trout. Pol. Arch. Hydrobiol. 3/4: 295-304.

Taggart, J., and A. Ferguson. 1984. An electrophoretically detectable genetic tag for hatchery reared brown trout, *Salmo trutta* L. Aquaculture 41: 119-130.

Taggart, J., and A. Ferguson. 1986. Electrophoretic evaluation of a supplemental stocking programme for brown trout *Salmo trutta* L. Aquacult. Fish. Manage. 17: 155-162.

# AN OVERVIEW OF SELECTION PRACTICES IN THE SALMON GENETICS RESEARCH PROGRAM

G. W. Friars, J. K. Bailey and F. M. O'Flynn[1]
Salmon Genetics Research Program
Atlantic Salmon Federation
P. O. Box 429
St. Andrews, New Brunswick E0G 2X0 Canada

[1] Graduate student at the University of New Brunswick, Saint John.

## ABSTRACT

Friars, G. W., J. K. Bailey, and F. M. O'Flynn. 1990. An overview of selection practices in the Salmon Genetics Research Program, p. 49-59. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

A mass-selection experiment for grilse length and a linear-selection-index experiment in two-sea-winter salmon are described. Correlated responses to selection for grilse length were observed in several of the covarying growth and developmental traits in fresh and seawater. However, estimates of correlations between family means suggested that multiple objective selection would be required to yield appreciable responses in performance in both fresh and seawater. Early results in the selection-index experiment also indicated positive correlated responses in certain juvenile traits.

## RÉSUMÉ

Friars, G. W., J. K. Bailey, and F. M. O'Flynn. 1990. An overview of selection practices in the Salmon Genetics Research Program, p. 49-59. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Le présent document décrit une expérience de sélection phénotypique pour déterminer la longueur des castillons ainsi que l'établissement d'un indice de sélection séquentielle chez le saumon de deux années en mer. Nous avons observé les réactions corrélées à la sélection pour la longueur des castillons, relativement à plusieurs caractères variables de croissance et de développement dans l'eau douce et dans l'eau salée. Cependant, l'estimation des correlations entre les moyennes familiales laisse supposer qu'une sélection objective multiple sera nécessaire pour donner des réactions appréciables au niveau du rendement, tant dans l'eau douce que dans l'eau salée. Les premiers résultats de l'expérience sur l'indice de sélection séquentielle indiquent également des réactions positives en ce qui a trait à certains caractères juvéniles.

## INTRODUCTION

The selection practices involved in the Salmon Genetics Research Program (SGRP) have been based on comparisons of control and select lines. The first of these comparisons involved mass selection for fork length after approximately 18 mo in sea cages as reported by Friars et al. (1990). Certain of the details of the correlated responses to selection, not reported in that paper, are included in this document.

The second experiment involved multiple objective selection where a linear index was employed. Estimates of the genetic, phenotypic, and economic parameters were assembled by Bailey (1988). Additional estimates of correlations between family means are reported here. Finally, some of the correlated response data for juvenile traits have been included in connection with the study involving the selection index.

## MASS SELECTION FOR LENGTH AT GRILSE AGE

A summary of the procedures used in this experiment were reported by Friars et al. (1990). The selection differential was 6.7 cm (1.52 $\sigma$) and there were no matings between individuals more closely related than first cousins.

### EGG AND FRY TRAITS

Comparisons of the egg and fry traits for the control and select lines (Table 1) reveal that the higher egg yield and superior survival of the select, compared to the control females, presents problems with respect to contrasts for the fry lengths and weights. Since entire families were reared in separate 1.1-m tanks, there were, on average, more fry per tank in the select than in the control line. The uncorrected means of progeny of the control were larger than those of the selected parents. The relationship between weight or length and the number of fish per tank is clearly demonstrated by the significant ($p < 0.05$) negative regression in Fig. 1. The homogeneity of the regression slopes in the control and select lines allowed a covariance adjustment for numbers of fish per tank. The uncorrected mean weights and lengths were larger for the controls than the selects. When corrected for stocking density per tank, the difference was reversed (Table 2). This result was paralleled by the fry-length data.

The inclusion of egg size as a second covariate (Table 2) reduced the apparent size difference between lines. The proportion of variance in the dependent variable, attributable to the covariates, was increased from 28 to more than 40% when egg size and numbers per tank were both used as covariates. This effect results from the fact that larger egg size is positively related to fry size, whereas larger families per individual tank affects fry size negatively. Hence, the larger families and larger eggs in the selected females, compared to those of the controls, are counteracting forces with respect to their effects on fry size.

### PARR AND SMOLT TRAITS

The fry were transferred to outside 7.6-m rearing tanks in June when the mean length was 5.58 cm. Stocking densities were adjusted and ranged between 2488 and 3399 individuals per tank. Three control and three select families were placed in each tank. Control families with large numbers of fry were approximately balanced, on a within-tank basis, with those that had smaller numbers. There were 300 fry of each of the three select families in each respective tank. Each set of six families was represented once in a fiberglass and once in a concrete tank. A total of 12 fiberglass and 12 concrete tanks were used.

The incidence of 1-yr-old (1+) smolts per family tank unit was evaluated during January and February. The demarcation point between presumptive smolts and parr was set at a fork length of 13 cm. A multiple regression of the effects of numbers of fish per fry tank and per parr tank on the proportion of presumptive smolt (Table 3), based on tank means, revealed that variation in stocking density in fry tanks had a greater effect than that in the parr tanks. This result was apparent even though the variance in smoltification rate, attributable to both of these covariates, amounted to only 4%. Hence, variables other than stocking density had large effects on the incidence of smolt.

The incidence of 1+ smolts, parr length, and smolt length all showed a positive correlated response to selection (Table 4). The analyses based on family means revealed a less striking difference than that based on strain-tank means. This result was attributable to the fact that some control families with large numbers, in contrast to small numbers per select family, showed a greater depression in the incidence of 1+ smolt in concrete as opposed to the fiberglass tanks.

## PERFORMANCE IN SEA CAGES

In the sea, each family was approximately divided between two sea cages. The superior performance of the select over the control line was apparent in the fork-length means (Table 5). The gain by 70 wk in seawater, about 2 mo prior to sexual maturity as grilse, was 1.84 cm. This strain was measured close to the age at which selection was applied and the realized heritability in the single generation selection experiment was 0.27.

## MULTIPLE OBJECTIVE SELECTION

The correlations between family means (Table 6) reveal that the pattern of relationships between freshwater and saltwater phases of performance are not consistent for the control and select lines. Although not always true, there was frequently a tendency for these correlations to get weaker the longer the fish were in seawater. The correlations indicate that selection for size in one of these phases of growth may not yield appreciable realized gains in the other phase.

## SELECTION PROCEDURES

In the light of the findings of Bailey (1988) which concur with those in Table 6, a multiple objective selection experiment was initiated in 1988 in a strain of marked and pedigreed Saint John River fish, which produced only 5.8% grilse. The parental population was subdivided into two run timing categories, early and late. Early-run salmon are defined as those appearing at the Mactaquac Fish Culture Station collection facility prior to September 1. Late-run salmon appear at the facility after that date. As in the earlier experiment, control and select lines were established in each category and traits of economic importance in fresh and seawater were selected using a linear index.

Parents of the control population were identified first. Where possible, one male and one female were chosen at random from each family. A small number of additional males and females was then picked at random from the entire population to produce a total of 100 unselected spawners (50 males and 50 females). The select parents were determined from the remaining broodstock on the basis of a selection index. The selection intensity was stronger in females where approximately 500 mature individuals were available, in contrast to only 300 males. The selection differential between the control and the selected parents was 0.8 standard deviations on the index scale.

The parameters used to derive the index equations and their interrelationships among traits were from Bailey (1988) and are illustrated in Fig. 2. The traits included in the index were fry length, percent 1+ smolts, harvest length (at about 2 mo before sexual maturity) and length at sexual maturity. The phenotypic observations for parr length and harvest length were deviations of the appropriate family means from the grand means. The observations were based on individuals in the case of percent 1+ smolts, where all members of the same family were assumed to have the same value. Both individual deviations from the family mean and family mean deviations from the grand mean were used for harvest length.

The selection procedure used during 1988 was designed to increase all four traits simultaneously. The greatest emphasis was placed on harvest weight, where that trait should yield the greatest comparative gain since it has the highest economic value. The economic weights and the expected relative genetic gains in the four traits in the index are illustrated in Fig. 2.

## CORRELATED RESPONSES

The fry from the control and select parents were sampled at approximately 3.5 mo after swim-up. Thirty fry from each family were measured for length and weight, where length was one of the freshwater traits used in the selection index. The results are presented in Table 7. While the select lines showed a small length and weight advantage over the controls, in both categories, the difference was significant ($P < 0.05$) in the case of the late-run subpopulation only. Fry length was given the lowest weight in the selection index and, therefore, only minimal gains were expected.

Although not included in the index, egg and survival traits including number of eggs per female, egg size, percent survival to the eyed egg stage, and percent survival from eyed egg to the fry stage were also monitored. The results are presented in Table 8. The differences between controls and selects were not significant ($P > 0.10$) for number of eggs per female and egg size. However, select fish of both early and late run parents had larger values than the controls for both traits. Thus, there may have been weak correlated responses to selection.

Data for survival were not normally distributed. The percentages for each family were used as point estimates and analyzed using the non-parametric Kruskal-Wallis analysis of variance and multiple comparisons tests for large samples. Control and select lines were not significantly different ($P > 0.10$). Again, the select values were superior to the control values, with the exception of survival to the eyed stage in the late run subpopulation (Table 8).

## PRIMARY BREEDER ROLE

The progeny of the selected grandparents will be distributed to multiplier growers. The parental fish at the multiplier grower sites will produce gametes that yield offspring to be distributed, through hatcheries, to commercial growers. The realized responses in the mass selection experiment and in the multiple objective selection experiment indicate that considerable economic gain can be expected.

## ACKNOWLEDGMENTS

The authors thank R. G. Carney and his staff for technical assistance. The data analyses of K. A. Coombs and C. G. Soto and the word processing of S. E. Young-Lai are acknowledged. Financial support for the research has been contributed by the Department of Fisheries and Oceans (Canada), the Atlantic Salmon Federation, and the New Brunswick Salmon Growers Association.

## REFERENCES

Bailey, J. K. 1988. Selection criteria for sea ranched and cage reared Atlantic salmon (*Salmo salar*). Ph.D. Thesis, University of Guelph.

Friars, G. W., J. K. Bailey, and K. A. Coombs. 1990. Correlated responses to selection for grilse length in Atlantic salmon. Aquaculture (in press).

Table 1. Means of control and select lines for numbers of fry per family, egg size, fork length, and weight at the fry stage (modified from Table 1 of Friars et al. (1990)).

| Trait | Control | Select |
|---|---|---|
| Eggs per female parent | 4430 | 5744[a] |
| Egg size ($mm^3$) | 94.1 | 100.0[a] |
| Survival to eyed stage (%) | 35.6 | 48.6[b] |
| Survival to fry stage (%) | 22.7 | 32.10[b] |
| Fry per family | 1270 | 2135[a] |
| Fork length (mm) | | |
| Uncorrected | 55.87 | 55.73 |
| Corrected | 55.14 | 56.28 |
| Weight (g) | | |
| Uncorrected | 2.07 | 1.99 |
| Corrected | 1.98 | 2.06 |

[a] Difference between control and select significant ($P < 0.01$).
[b] Difference between control and select significant ($P < 0.05$).

Table 2. Fry weight (g) and length (cm) after correction for stocking density (numbers per 1.1-m tank) and egg size ($mm^3$). Covariates are in parentheses. Analyses performed on tank means.

| Trait | Control | Selected | Probability[a] | Proportion of variance[b] |
|---|---|---|---|---|
| Fry weight (stocking density) | 1.97 | 2.06 | 0.19 | 0.34 |
| Fry length (stocking density) | 5.51 | 5.63 | 0.06 | 0.29 |
| Fry weight (stocking density and egg size) | 2.03 | 2.03 | 1.00 | 0.46 |
| Fry length (stocking density and egg size) | 5.56 | 5.60 | 0.51 | 0.41 |

[a] Probability of a type 1 error.
[b] Proportion of variance in the dependent variable attributable to covariate(s).

Table 3. Multiple linear regression of proportion of larger grade (>13 cm) at 12-14 mo posthatch on stocking densities based on tank means.

| Parameter | Partial regression coefficient | $P(b_x)=0$ |
|---|---|---|
| $b_0$, intercept | 0.57 | 0.0001 |
| $b_1$, number/fry tank | $-3.08 \times 10^{-5}$ | 0.0106 |
| $b_2$, number/parr tank | $5.43 \times 10^{-5}$ | 0.2608 |
| $R^2 = 0.04$ | | |

Table 4. Percent smolts and mean fork lengths of Atlantic salmon smolt and parr (adapted from Friars et al. 1990).

| Trait | Control | Select |
|---|---|---|
| Percent 1+ smolt | | |
| Tank model[a] | 62.20 | 69.10[b] |
| Family model | 67.23 | 69.51 |
| Fork length (mm) | | |
| Smolt | 16.21 | 16.48[c] |
| Parr | 11.05 | 11.15[c] |

[a] In the tank model the means of three control and of three select families were point estimates, whereas family means were used in the family model.

[b] Difference between control and select significant ($P < 0.05$).

[c] Difference between control and select significant ($P < 0.01$).

Table 5. Least square mean fork lengths (cm) of Atlantic salmon reared in sea cages (adapted from Friars et al. 1990).

| | Weeks in seawater | | | |
|---|---|---|---|---|
| Line | 13 | 27 | 54 | 70 |
| Control | 28.82 | 44.20 | 53.80 | 66.65 |
| Select[a] | 29.31 | 45.35 | 55.09 | 68.49 |

[a] Select line significantly larger than the control ($P < 0.01$) at all ages.

Table 6. Correlations between family means of fresh and seawater traits.

| Fork length/ weeks in seawater | Freshwater traits | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control line | | | | Select line | | | |
| | Adjusted fry length | Parr length | Smolt length | Percent smolt | Adjusted fry length | Parr length | Smolt length | Percent smolt |
| 13 | 0.34 | 0.23 | 0.60[a] | 0.42[a] | 0.26 | 0.25 | 0.52[a] | 0.06 |
| 27 | 0.15 | 0.37[b] | 0.30 | 0.20 | 0.10 | 0.42[a] | 0.49[a] | -0.12 |
| 70 | 0.09 | 0.00 | -0.07 | 0.08 | 0.23 | 0.49[a] | 0.36[a] | 0.17 |

[a] Significantly different from zero ($P < 0.01$).
[b] Significantly different from zero ($P < 0.05$).

Table 7. Least sqares means of fry weight and fork length (corrected for number per tank) of progeny from parents of a control line and a line selected on an index involving fresh and seawater traits of two-sea-winter salmon.

| Trait | Early run | | Late run | |
|---|---|---|---|---|
| | Control | Select | Control | Select |
| Length (cm) | 6.34 | 6.49 | 6.46 | 6.80[a] |
| Weight (g) | 2.78 | 2.98 | 3.08 | 3.62[a] |

[a] Difference between select and control significant at $P < 0.05$.

Table 8. Egg and survival traits of eggs from a control line and a line selected on an index involving fresh and saltwater traits of two-sea-winter salmon.

| Trait | Early run | | Late run | |
|---|---|---|---|---|
| | Control | Select | Control | Select |
| Eggs/female | 14,594 | 15,258 | 14,343 | 15,513 |
| Egg size (mm$^3$) | 102.68 | 107.30 | 106.83 | 108.78 |
| Early survival[a] (%) | 47.5 | 57.7 | 65.6 | 48.4 |
| Late survival[b] | 23.3 | 46.0 | 40.5 | 59.5 |

[a] Measured from the green egg to the eyed stage.
[b] Measured from the eyed egg to the fry stage.

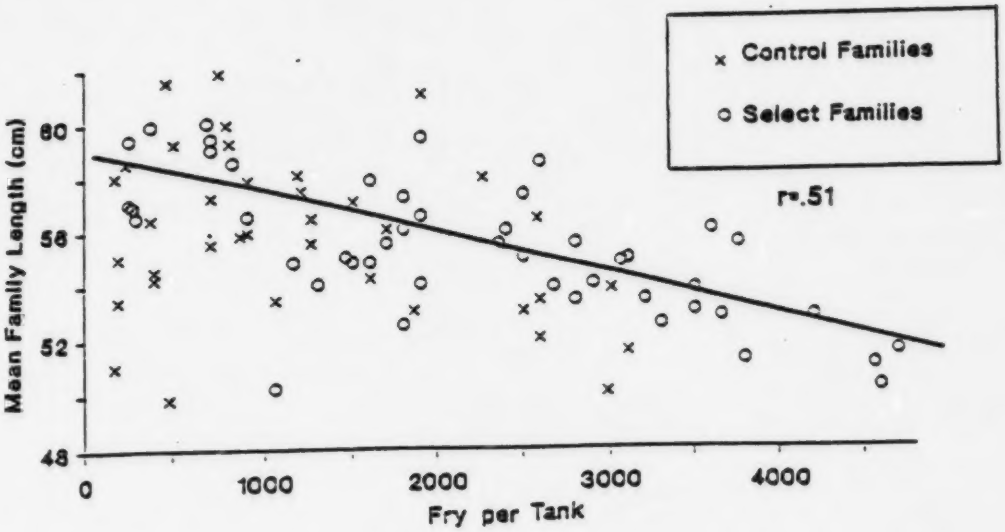


Fig. 1. Regression of fry weight on number of fry per tank.

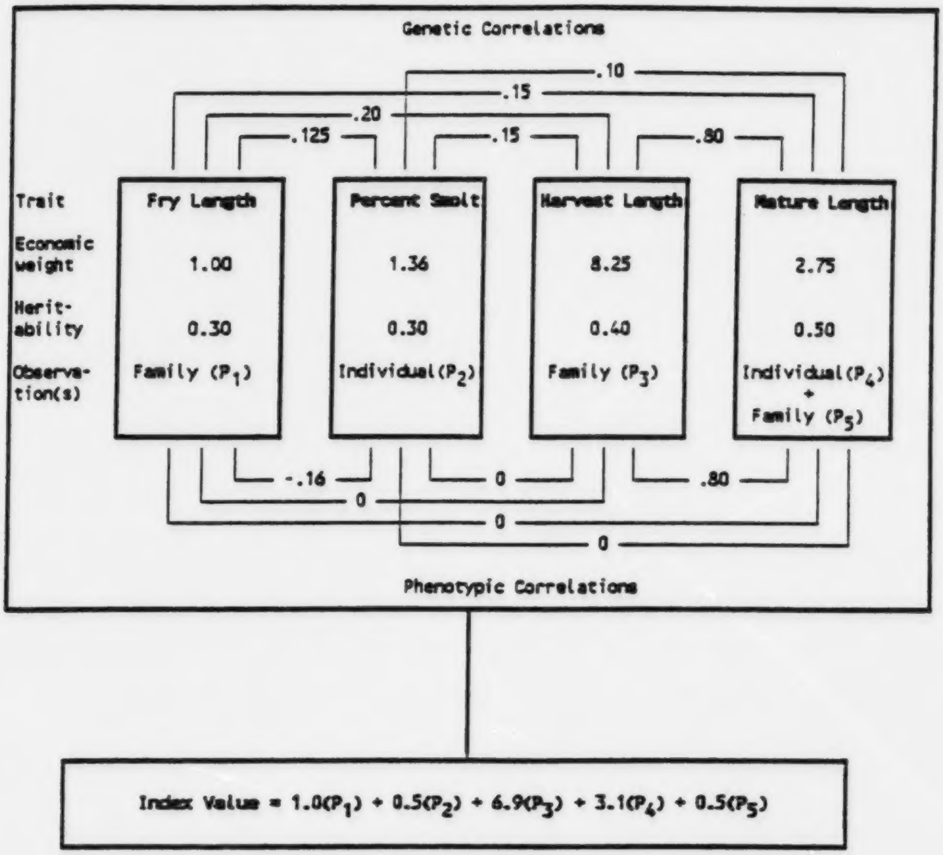

Genetic Correlations
.10
.15
.20
.125
.15
.80
Trait
Economic weight
Heritability
Observation(s)
Fry Length
1.00
0.30
Family (P1)
Percent Smolt
1.36
0.30
Individual(P2)
Harvest Length
8.25
0.40
Family (P3)
Mature Length
2.75
0.50
Individual(P4) + Family (P5)
-.16
0
.80
0
0
0
Phenotypic Correlations
Index Value = 1.0(P1) + 0.5(P2) + 6.9(P3) + 3.1(P4) + 0.5(P5)

Fig. 2. Traits and the phenotypic and genetic parameters used to generate the selection index.

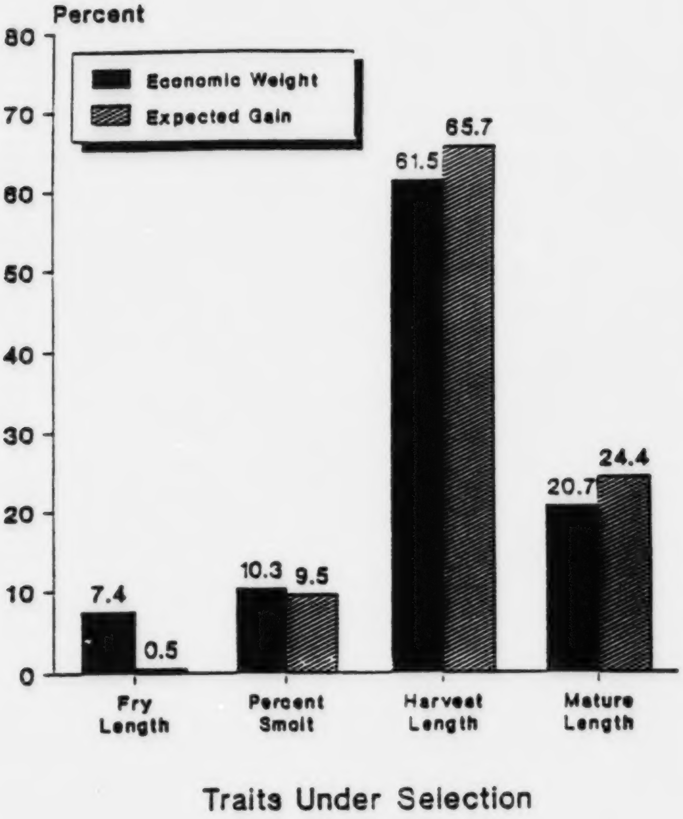


Fig. 3. Relative economic weights and percent of expected genetic gain for each trait in the selection index.

# TANK EFFECTS AS CONFOUNDING FACTORS IN GENETIC EXPERIMENTS: EXPERIENCES WITH ARCTIC CHARR (*SALVELINUS ALPINUS*)

B. G. E. de March
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6 Canada

## ABSTRACT

de March, B. G. E. 1990. Tank effects as confounding factors in genetic experiments: experiences with Arctic charr (*Salvelinus alpinus*), p. 61-68. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

This manuscript describes initial stages in the development of a deterministic model which describes the mean and variance of growth in tank populations.

Several experiments with Arctic charr (*Salvelinus alpinus*) examining the genetic basis of growth characters suggested that within-tank means and variances were partly determined by initial means and variances and the consequent social structure. One experiment with 24 tanks of Arctic charr reared from hatching to approximately 1 yr is presented to exemplify the tank effects. The variability in different tanks of full-sib families, expressed as a CV, changed only slightly during the course of the experiment. This variability was primarily a dam effect, but also due to chance when allocating fish to replicate tanks. Mean weights of offspring at 30 and 75 d were related to both sires and dams. After approximately 100 d, tanks of fish with large initial coefficients of variation (CV) increased in mean weight less rapidly than those with small CV's. At 275 d, the mean weights in tanks were more strongly and negatively correlated with CV's than with mean weights measured early in the experiment.

It was felt that correcting for covariates such as initial sizes, density, or time of spawning was not sufficient for comparing tank populations since individuals' expression of genotype depended on the group structure in the tank. Theoretically justified correction factors describing relevant aspects of the group structure would also be desirable.

Various growth models for individuals in groups were examined to determine how these relationships might develop in tanks with different initial conditions, and how these initial conditions might be expressed in linear models. Progress in these investigations is reported.

## RÉSUMÉ

de March, B. G. E. 1990. Tank effects as confounding factors in genetic experiments: experiences with Arctic charr (*Salvelinus alpinus*), p. 61-68. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Ce résumé expose les premières étapes de l'élaboration d'un modèle déterministe des tendances observées dans la croissance des populations en cuves.

Plusieurs expériences pour examiner le fondement génétique des caractéristiques de croissance de l'omble chevalier (*Salvelinus alpinus*) semblaient indiquer que les moyennes et les variances observées à l'intérieur des cuves étaient partiellement déterminées par les moyennes et les variances de départ et par la structure sociale qui y est associée. Pour illustrer l'effet de cuve, on

expose les résultats d'une expérience réalisée à partir de 24 cuves d'ombles chevaliers d'un an environ en élevage depuis l'éclosion. La variabilité de différentes cuves de familles de pleins germains, exprimée en coefficient de variation (c.v.), ne changeait que légèrement au cours de l'expérience. Cette variabilité était surtout due aux mères mais aussi au hasard, au moment de la répartition des poissons dans des cuves identiques. Le poids moyen de la progéniture à 30 et 75 d a été corrélé avec les mères et les pères. Après environ 100 d, les poissons des cuves dont les coefficients de variation (c.v.) étaient importants au départ ont vu leur poids moyen augmenter moins rapidement que ceux des cuves à faible c.v. À 275 d, le poids moyen des poissons était en corrélation plus étroite et négative avec le c.v. qu'avec le poids moyen déterminé au début de l'expérience.

On estimait que le rajustement des covariables telles les dimensions initiales, la densité ou le moment du frai n'était pas suffisant pour comparer les populations des cuves, car les expressions individuelles du génotype dépendaient de la structure du groupe dans la cuve. Il serait également préférable de disposer de facteurs de correction justifiés théoriquement, décrivant les aspects pertinents de la structure du groupe.

On a examiné divers modèles de croissance pour les sujets des groupes afin de déterminer comment ces rapports peuvent se développer dans les cuves dont les conditions de départ sont différentes et comment ces conditions de départ peuvent être exprimées en modèles linéaires. On signale des progrès dans ces recherches.

## INTRODUCTION

Experiments in which fish growth is monitored are confounded by the fact that fish must be reared in groups and in a restricted environment. In charr, either too low or too high densities in a tank decreases the growth of individuals (Baker 1983). Corrections for environmental or initial conditions can be made by their inclusion as covariates in linear models, for example, as was done by Refstie et al. (1977), Refstie and Steine (1978) and Refstie (1980). It is reasonable that if individuals have different probabilities of expressing their genotypes due to the group structure, correction factors expressing group structure should also be considered.

The design and summary results of one experiment with juvenile Arctic charr (*Salvelinus alpinus*) of stock originally from Labrador, Canada, in which normal growth was monitored from hatching to approximately 1 yr, are shown in Table 1 and Table 2. More details of the sampling methodology are given in de March (in prep.); sufficient raw data are given here to support generalizations. Growth in each tank was described by a logistic growth curve, that is,

$$\text{Mean weight} = A/(1+e^{-k(t-t_o)}),$$

in which A is the predicted mean asymptote, k the predicted growth rate when the weight is zero and $t_o$ the inflection point of the sigmoid curve in days (Ricker 1975). Table 1 shows A and k and the weights at 30, 75, 125 and 275 d in all 24 tanks. The variability, expressed as a coefficient of variation (CV = 100*standard deviation/mean), was measured when the mean weight in each tank was near 10 g and again when the mean weight was near 60 g (Table 1). Both dam and sire effects are evident for weights at 30 and 75 d and for the predicted asymptote (de March, in prep.) (Table 2). The mean weight at 275 d was also related to the parents, but the relationship of particular female parents to offspring size had changed (Table 2). The underlying factor in the changes seemed to be the CV's - mean weights increased more slowly in tanks with high CV's than in tanks with low CV's. The offspring of dam F3, and perhaps sire M4, had very high CV's and low final weights; however, if these are not considered, the same generalization can still be made (Table 1 (de March, unpubl. data)).

Similar phenomena have been reported, but not analyzed, by other authors. Brown (1946) believed that stock densities affected the initiation of hierarchies and that the size of individuals determined their position in a hierarchy. This hierarchy accounted for differential growth of fish and the fact that the CV changed little. Purdom (1974) believed that "part of the variance can be attributed to the hierarchy." The results of the described experiment and others with charr suggest that the manner in which this hierarchy manifests itself may be dependent on initial weights, initial variances and the strain of fish.

The object of the described modelling exercise was 1) to determine what type of growth processes in individuals might relate the described temporal trends, and 2) to determine whether simple covariates can be used to correct for social interactions within tanks. Simulated populations with the different initial mean values and CV's were generated, simple hypotheses about the growth of individual fish were made, simulated populations were allowed to "grow" and the resultant trends were compared to experimental results. Alternative hypotheses were examined with attention to conceptual simplicity, adaptability to statistical linear models, and adaptability to the results of different experiments.

## METHODS

The approximately normally distributed weights at 75 d of 11 individuals within populations (i=1,11) were generated by calculating $Wt_{oi} = SD_o*BLOM_i + MWt_o$, in which $MWt_o$ is the chosen initial mean weight in the population, $SD_o$ the chosen initial standard deviation and $BLOM_i = \text{probit}((X_i - 3/8)/(11.25))$, in which $X_i$ is the rank order (1 to 11) of the individuals (Tukey 1962). The initial weights at 75 d and the CV's were similar to those in the described experiment.

The growth of all individuals was assumed to follow a logistic curve. If curves are assumed to be vertically parallel, these can be expressed in terms of the weight at 75 d rather than of $t_o$ as follows:

$$Wt_o = A_i/(1+\underline{e}^{-k_i(t-75)}(A_i-Wt_{oi})/Wt_{oi}) \qquad [1]$$

in which $A_i$ is the asymptote, $k_i$ the rate, and $Wt_{oi}$ the initial weight of fish i. Fish in the simulated populations "grew" according to this equation after different hypotheses about and between the parameters $A_i$ and $k_i$ were made.

Table 1. Results of factorial mating design crosses (4 Females x 4 Males) with Labrador charr. n = final number of fish per tank (initial numbers are 50, 100 and 150). Wt 30 d = the mean weight of fish measured as a batch weight 30 d after swim-up. This measurement was obtained only once before each family was split into several tanks. A and k are parameters from the logistic growth describing the mean weight in each tank, Wt(g) 75, 125 and 275 d are mean weights on specific days. CV's were measured twice, once when the mean weight in a tank was near 10 g and again when it was near 60 g. "Rep" represents replicate tanks from the same cross.

| Cross | n | Wt(g) 30 d | A | k | Wt(g) 75 d | Wt(g) 125 d | Wt(g) 275 d | CV(%) 10 g | CV(%) 60 g |
|---|---|---|---|---|---|---|---|---|---|
| F4 x M3 | 43 | 0.13 | 70.7 | 0.025 | 1.04 | 3.53 | 49 | 53 | 53 |
| F4 x M1 | 49 | 0.13 | 83.0 | 0.027 | 1.29 | 4.70 | 64 | 46 | 47 |
| F4 x M2 | 48 | 0.14 | 76.8 | 0.026 | 1.48 | 5.17 | 60 | 47 | 40 |
| F2 x M1 | 47 | 0.15 | 73.9 | 0.027 | 0.94 | 3.54 | 55 | 36 | 26 |
| Rep | 98 |  | 78.2 | 0.027 | 0.91 | 3.37 | 56 | 39 | 28 |
| Rep | 141 |  | 77.5 | 0.025 | 0.85 | 2.82 | 46 | 44 | 32 |
| F1 x M1 | 49 | 0.15 | 75.1 | 0.030 | 0.96 | 4.10 | 63 | 38 | 31 |
| Rep | 50 |  | 85.2 | 0.023 | 1.14 | 3.58 | 51 | 49 | 38 |
| Rep | 95 |  | 67.4 | 0.027 | 1.05 | 3.87 | 52 | 48 | 40 |
| F1 x M4 | 40 | 0.16 | 63.6 | 0.026 | 0.94 | 3.28 | 46 | 74 | 79 |
| Rep | 81 |  | 74.5 | 0.027 | 1.09 | 3.93 | 56 | 44 | 50 |
| F1 x M3 | 49 | 0.17 | 70.4 | 0.025 | 0.95 | 3.19 | 47 | 43 | 36 |
| Rep | 89 |  | 61.4 | 0.026 | 0.90 | 3.19 | 45 | 38 | 40 |
| Rep | 143 |  | 65.2 | 0.023 | 0.91 | 2.84 | 39 | 45 | 38 |
| F3 x M1 | 46 | 0.17 | 99.6 | 0.020 | 1.22 | 3.29 | 42 | 70 | 60 |
| F2 x M2 | 49 | 0.19 | 78.9 | 0.027 | 0.95 | 3.47 | 56 | 51 | 45 |
| Rep | 93 |  | 76.5 | 0.028 | 0.84 | 3.30 | 57 | 37 | 33 |
| Rep | 148 |  | 85.1 | 0.025 | 0.94 | 3.15 | 52 | 34 | 26 |
| F1 x M2 | 48 | 0.20 | 75.7 | 0.028 | 1.34 | 5.08 | 62 | 39 | 34 |
| Rep | 95 |  | 79.1 | 0.026 | 1.19 | 4.15 | 57 | 33 | 34 |
| Rep | 146 |  | 75.1 | 0.023 | 1.16 | 3.58 | 46 | 38 | 36 |
| F3 x M3 | 48 | 0.22 | 87.1 | 0.022 | 1.26 | 3.71 | 48 | 59 | 48 |
| F3 x M2 | 47 | 0.23 | 99.1 | 0.020 | 1.68 | 4.47 | 49 | 71 | 67 |
| F3 x M4 | 49 | 0.28 | 95.8 | 0.020 | 1.77 | 4.57 | 47 | 55 | 46 |

Table 2. Summary of analysis of covariance of data on Table 1. The model $Y = n + n^2 + d_i + s_j$, in which Y are mean weights or CV's from Table 1, n the number of fish in each tank when the parameter was measured, $d_i$ the effects of dam i, and $s_j$ the effects of each sire j, was fitted using the PROC GLM (General Linear Models Procedure) in SAS (SAS Institute 1985). The joint probability of the significance of n and $n^2$ are assessed with the ESTIMATE option. Expected mean values for each parent, calculated by holding male effects at the mean value to calculate the effects of different female parents and visa-versa, were obtained with the Least Squares Mean Option (LSMEANS). All probabilities were calculated from partial or Type IV sums of squares.

| | Dependent variable | | | |
|---|---|---|---|---|
| | Wg(g) | Wt(g) | CV | |
| | 75 d | 275 d | 10 g | 60 g |
| Probability of effects | | | | |
| n and $n^2$ | 0.97 | 0.17 | 0.18 | 0.43 |
| Dam | 0.00 | 0.04 | 0.10 | 0.34 |
| Sire | 0.06 | 0.02 | 0.54 | 0.25 |
| Model $R^2$ | 0.85 | 0.71 | 0.61 | 0.56 |
| Expected mean values for parents | | | | |
| F1 | 0.06 | 51 | 46 | 43 |
| F2 | 0.85 | 52 | 44 | 37 |
| F3 | 1.48 | 45 | 61 | 52 |
| F4 | 1.28 | 56 | 48 | 47 |
| M1 | 1.15 | 53 | 48 | 40 |
| M2 | 1.31 | 56 | 46 | 42 |
| M3 | 0.99 | 46 | 47 | 42 |
| M4 | 1.23 | 50 | 56 | 56 |

## RESULTS

Hypotheses tested, in increasing order of complexity, and conclusions drawn from the simulated data were as follows:

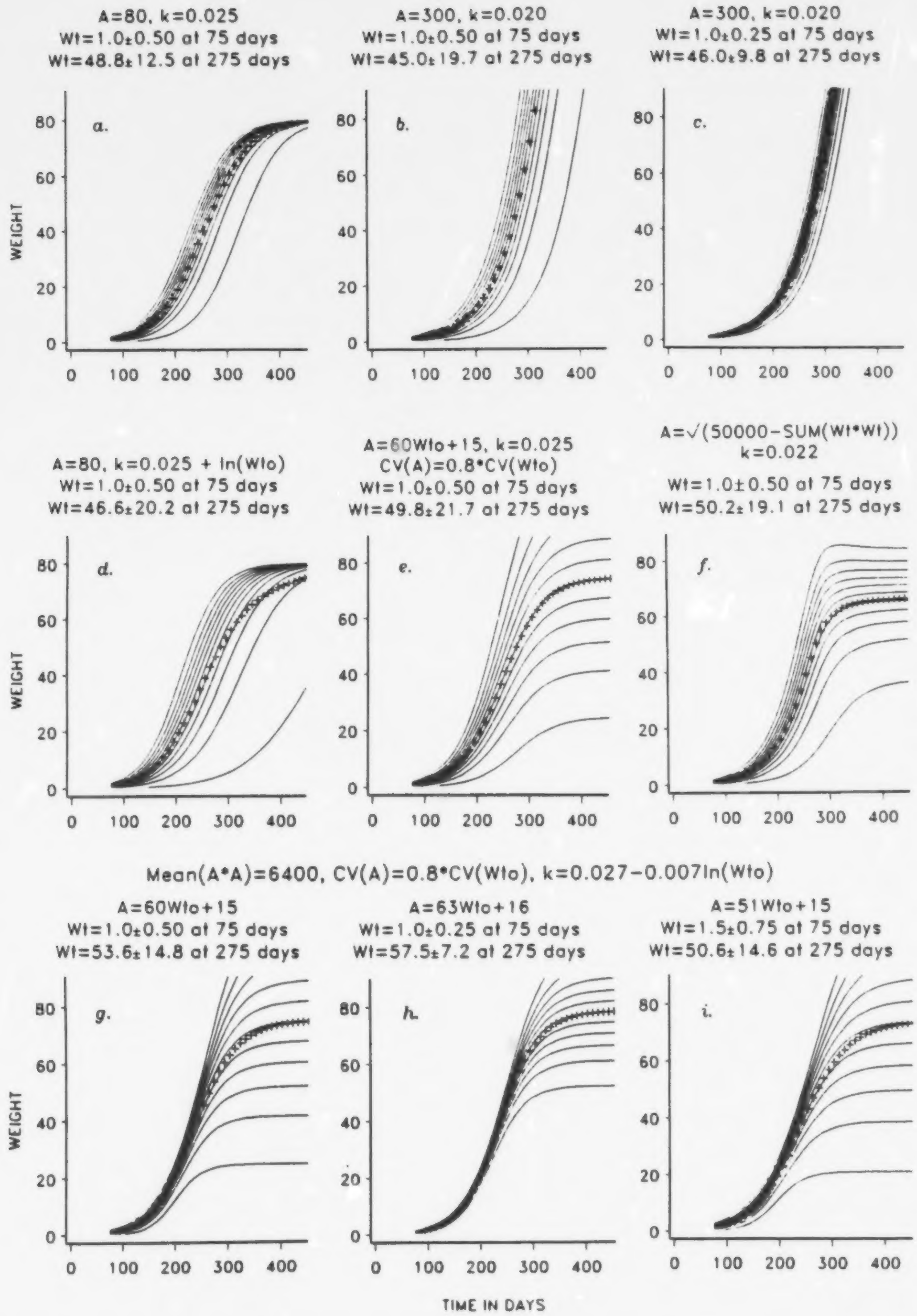
### HYPOTHESIS 1

All fish of different initial weights within and between tank populations follow the same vertically parallel logistic growth curves (Equation [1]), that is, have identical values of $A_i$ and $k_i$. The variation in initial weights are the sole cause of different mean growth rates between and size variability within tanks.

If A = 80 g and k = 0.025 (close to the mean value from Table 1), and the initial mean weight and CV are 1.0 ± 0.5 g (50%), then the mean weight and CV at 275 d are 48.8 ± 12.5 g (26%) (Fig. 1a). Another population (not shown) with initial values of 1.0 ± 0.25 g (25%) increased to 51.8 ± 3.8 g (7.3%) at 275 d. There is a slight inverse relationship between mean weight and the CV on a given date, entirely due to the initial CV. The difference in mean weights due to the initial CV is similar to experimental populations, but the decrease in CV's as individual weights all approach the same asymptote is not realistic.

It is possible that the true asymptote during most of the experiment is actually much larger than 80 g, and that some sort of limiting conditions occurred late in the experiment (50 fish per tank are quite easily grown to a mean weight of 300 g in less than 2 yr). Figures 1b and 1c show the growth with A = 300, k = 0.020, and the same initial mean weights and CV's as above. In the simulated data, the initial variation changes only slightly, as in the experimental population. However, the difference between the mean weights due to initial CV's is much smaller than in the real data.

Other choices of A and k applied to populations with different initial values also did



A=80, k=0.025
Wt=1.0±0.50 at 75 days
Wt=48.8±12.5 at 275 days

A=300, k=0.020
Wt=1.0±0.50 at 75 days
Wt=45.0±19.7 at 275 days

A=300, k=0.020
Wt=1.0±0.25 at 75 days
Wt=46.0±9.8 at 275 days

A=80, k=0.025 + ln(Wto)
Wt=1.0±0.50 at 75 days
Wt=46.6±20.2 at 275 days

A=60Wto+15, k=0.025
CV(A)=0.8*CV(Wto)
Wt=1.0±0.50 at 75 days
Wt=49.8±21.7 at 275 days

A=√(50000-SUM(Wt*Wt))
k=0.022
Wt=1.0±0.50 at 75 days
Wt=50.2±19.1 at 275 days

Mean(A*A)=6400, CV(A)=0.8*CV(Wto), k=0.027-0.007ln(Wto)

A=60Wto+15
Wt=1.0±0.50 at 75 days
Wt=53.6±14.8 at 275 days

A=63Wto+16
Wt=1.0±0.25 at 75 days
Wt=57.5±7.2 at 275 days

A=51Wto+15
Wt=1.5±0.75 at 75 days
Wt=50.6±14.6 at 275 days

**Figure 1. Simulated logistic growth curves generated by considering various growth curve options. The +'s indicate the trend in the mean value. Initial weights at 75 d were generated as described in the manuscript. $Wt_o$ refers to individual initial weights, Wt is the mean weight in a tank.**

not simulate the real data. As exemplified by Fig. 1a-1c, the inverse relationship between final weights and CV's were too weak and/or the CV's changed too much.

## HYPOTHESIS 2

All individuals, regardless of tank population structure, have asymptotes ($A_i$) or rates ($k_i$) dependent on their initial weights. Fish with different initial weights grow differently but independently of each other. These relationships account for the differences in mean tank growth rates and variability within tanks.

If the assumption that $k_i$ is related to $Wt_{oi}$ is made, then tank populations still converge to an asymptote (Fig. 1d). This is not a desirable modelling option on its own.

The assumption that each fish's asymptotic value, $A_i$, is a linear function of the initial weight, that is, $A_i = X^*Wt_{oi} + Y$, is a practical modelling alternative for several reasons: 1) individuals do not reach the same asymptote and the variation is not necessarily reduced with time (Fig. 1e); 2) a variety of relationships between the initial and final variances can be described. With the above linear relationship, the mean asymptotic value is $A = X^*MWt_o + Y$, the variance of the asymptote is $X^{2*}Var_o$, and the CV of this asymptote is

$$CV(A) = 100^*(X^*SD_o)/(X^*MWt_o + Y) \qquad [2],$$

in which $Var_o$, $SD_o$ and $MWt_o$ are the initial variance, standard deviation and mean weight, respectively. A desired change in the CV can be modelled by the choice of X and Y. In the experiment presented here, the CV changes little but, in others, steady increases or decreases with time have been observed; 3) this assumption gives theoretical justification for the use of initial mean weights as a correction factor in a linear model.

Simulations under this hypothesis were similar to, and had the same inadequacies as, those under Hypothesis 1. The increase correlation between the final mean weight and the initial CV's for a range of initial means could not be attained.

It can be concluded that the observed results are not due to fish growing independently of each other. Models which include the initial means or variability must be considered.

## HYPOTHESIS 3

A "tank asymptote," defined in terms of both total weight and variation, exists.

The possibility of a simple "tank asymptote" became evident when I truncated the growth in simulated populations. For example, with A = 300 and k = 0.020, if growth was truncated when the total uncorrected sums of squares (SUM(Weight$^2$)) was a constant, 50000 in this case, then final mean weights were more strongly and negatively correlated with CV than with initial weights. There is no evidence of sudden truncation in the experiment, but it is not impossible that asymptotic values might develop by a similar process. Viewing the summary data on Table 1 in terms of uncorrected sums of squares in each family gives some support to the possibility that $Wt_2$ is an important dependent variable. The weights of offspring from Female 3 and Male 4, for example, have relatively large uncorrected sums of squares throughout time, first by having relatively large early weights, and then by having a high variance.

It is possible that the asymptote for each individual changes as the population grows and the tank asymptote is approached. To test this hypothesis, the growth curves in Fig. 1f were generated by giving each individual an asymptote of $A_i$ = SQRT(50000-SUM(Weight$_i^2$)), in which SUM(Weight$_i^2$) is the uncorrected sum of squares of weights for all other individuals on each day, and then allowing 1 d growth. $A_i$ is thus one expression of the amount of "territory" left in a tank. The mean asymptote was ca. 224 g at 75 d and ca. 65 g when growth ceased. This model did not generate growth curves that were responsive to the initial CV.

Many other options are possible in this type of iterative model, but these were not explored because a linear model is more desirable.

## ONE POSSIBLE GROWTH MODEL

One attempt was made to combine the desirable features of Hypotheses 2 and 3 into a linear model. The following options were chosen: 1) asymptotes for individuals were proportional to the initial weights, namely $A_i = X^*Wt_{oi} + Y$; 2) asymptotes were chosen to that T = SUM($A_i^2$) = 70400. Since for each individual i, $A_i = X^*Wt_{oi} + Y$, and the tank asymptote T = SUM($A_i^2$), it can be shown that:

$$A_i = X^*Wt_{oi} - X^*Wt_o + SQRT(T/n\text{-}X^{2*}Var_o) \quad [3].$$

Thus $Var_o$ is legitimately part of the growth curve describing the mean; 3) X and Y were chosen, with inclusion of Equation [2] in the calculations, so that final predicted CV of the mean asymptote was 80% of the initial; 4) individual $k_i$'s were chosen to be inversely proportional to initial weights. This last choice was made because I believe that smaller fish stopped growing sooner.

Three growth curves with different initial means and CV's are shown in Fig. 1g, h and i. In fact, the final relationship between the final means and the variance is similar to experimental data.

## DISCUSSION

A final model which can correct for the social climate in different tanks has not been chosen, but some of the more promising modelling options are demonstrated. Essentially, any model which has two different functions of an individual's initial weight and, particularly if one function includes the mean value, contains an expression of the variation. Final decisions about choices of correction factors and forms of dependent variables best used in the statistical analysis of experiments in which tank effects occur will depend on data from a larger diversity of experiments and larger and more accurate simulations, perhaps also involving skewed and flattened distributions.

The original manuscript with the data (de March, in prep.) used CV as a linear correction factor to describe the social climate in tanks. The results of these simulations suggest that this decision was not only practical, but may be theoretically justified.

Arctic charr are considered to be uniquely paedomorphic and a plastic species in the wild (Balon 1984), this plasticity perhaps expressed as confounding tank effects in experiments and lack of control of size variability in aquaculture. Although the tank effects may be extreme in Arctic charr, an understanding of the underlying factors may be important for the culture of any schooling species.

## ACKNOWLEDGMENTS

Many thanks to D. Bowes and R. Olson from the Rockwood hatchery who assisted with the spawning and maintenance of the fish.

## REFERENCES

Baker, R. 1983. The effects of temperature, ration and size on the growth of Arctic charr (*Salvelinus alpinus* L.). M.Sc. Thesis, Univ. of Manitoba, Winnipeg, MB, 227 p.

Balon, E. K. 1984. Life histories of Arctic charrs: an epigenetic explanation of their invading ability and evolution. In L. Johnson and B. L. Burns (ed.) Biology of the Arctic charr. Proc. Intern. Symp. Arctic Charr, Winnipeg, Manitoba, May 1981. University of Manitoba Press, Winnipeg, Canada.

Brown, M. E. 1946. The growth of brown trout (*Salmo trutta*). II. The growth of two-year-old trout at a constant temperature of 11.5°C. J. Exp. Biol. 22: 130-144.

Purdom, C. E. 1974. Variation in fish, p. 347-355. In F. R. H. Jones (ed.) Sea Fisheries Research. Paul Elek Ltd., Halstead Press, London, England.

Refstie, T. 1980. Genetic and environmental sources of variation in body weight of rainbow trout fingerlings. Aquaculture 19: 351-357.

Refstie, T., and T. A. Steine. 1978. Selection experiments with salmon. III. Genetic and environmental sources of variation in length and weight of Atlantic salmon in the freshwater phases. Aquaculture 14: 221-234.

Refstie, T., T. A. Steine, and T. Gjedrem. 1977. Selection experiments with salmon. II. Proportion of Atlantic salmon smoltifying at 1 year of age. Aquaculture 10: 231-242.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, North Carolina, 956 p.

Sparre, P. 1976. A Markovian decision process applied to optimization of production planning in fish farming. Meddr. Danm. Fish. -og Havunders 7: 111-197.

Tukey, J. W. 1962. The future of data analysis. Ann. Math. Stats. 33: 22.

# ARTIFICIAL HATCHING SUBSTRATES IMPROVE GROWTH AND YOLK ABSORPTION OF SALMONIDS

Tom Hansen, Rune Christiansen, Ragnar Nortvedt,
Sigurd Stefansson and Geir Lasse Taranger
Institute of Marine Research
Matre Aquaculture Research Station
N-5198 Matredal, Norway

## ABSTRACT

Hansen, T., R. Christiansen, R. Nortvedt, S. Stefansson, and G. L. Taranger. 1990. Artificial hatching substrates improve growth and yolk absorption of salmonids, p. 69-75. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Artificial hatching substrates eliminate the falling and righting which is associated with incubation of Atlantic salmon alevins in flat screen incubation systems. The consequence is a reduction in swimming activity and a higher growth rate of alevins incubated in hatching substrates. The flat-screen incubated alevins also have reduced yolk absorption rate compared with those incubated on substrate, probably as a consequence of high activity of the alevins. The hatching substrates also resolve the problems with yolk sac constriction, a condition which is maintained and aggravated by high activity of alevins.

## RÉSUMÉ

Hansen, T., R. Christiansen, R. Nortvedt, S. Stefansson, and G. L. Taranger. 1990. Artificial hatching substrates improve growth and yolk absorption of salmonids, p. 69-75. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'éclosion dans des substrats artificiels élimine les tendances aux chutes et aux redressements qui sont associées à l'incubation des alevins de saumon de l'Atlantique dans des caissons d'incubation plats. Il en résulte une réduction de l'activité natatoire et un taux de croissance plus élevé des alevins qui éclosent dans des substrats d'éclosion. Les alevins qui éclosent dans des caissons d'incubation plats connaissent un taux de résorption du sac vitellin moins élevé que les alevins qui éclosent dans des substrats, et ce, probablement à cause de la grande activité des alevins. L'élevage dans les substrats d'éclosion résoud aussi le problème de la constriction du sac vitellin, une situation qui est entretenue et aggravée par le taux d'activité élevé des alevins.

## INTRODUCTION

Several different types of incubators have been developed for incubation and hatching of salmonids. In Norway, the 'California' type incubator is the most popular system (Edwards 1978). However, Norwegian hatcheries have experienced high mortalities, both during incubation and first feeding, using this type of incubator. As a consequence, we started the project "Hatching-fry quality" in 1982. The aim of this project was to examine the environment within the incubation system and determine the factors necessary for the production of good quality fry. The project has clearly demonstrated the negative effect, during culture, of not taking into consideration the adaptations of the species to its natural environment. This paper presents some of the results from this project, discussing them in relation to available scientific literature.

## BEHAVIOR

Bams (1969) and Marr (1963) discovered and described a behavioral response called the righting response, ensuring maintenance of an upright position of the salmonid alevin. This response has been described for sockeye salmon (*Oncorhynchus nerka*) by Bams (1969), brown trout (*Salmo trutta*) by Marr (1963), Atlantic salmon (*Salmo salar*) by Hansen (1984) and Nortvedt (1987), and rainbow trout (*O. mykiss*) by Nortvedt (1987). There are conspicuous differences in righting behavior of alevins incubated in hatching trays, either with or without a hatching substrate. Differences in activity, the positioning of the alevins in the tray, and their response to physical stimuli (e.g. light) are related to the righting response.

Marr (1963) observed that the falling and righting of alevins reared on a plain surface were compensated by high swimming activity which stabilized the alevins in the vertical plane. This increase in activity has been confirmed by Bams (1969), Leon (1975), Eriksson and Westlund (1983), Hansen and Møller (1985), and by Nortvedt (1987).

Nortvedt (1987) compared and quantified the behavior of Atlantic salmon and rainbow trout alevins incubated in Astroturf (ATR) and flat, screened hatching trays (FSR). A recalculation of his data show that ATR and FSR salmon alevins incubated in darkness (6.8°C) swam distances of 41.7 and 302.4 m, respectively, during the period from hatching until swimup (day 50). During this period, the FSR alevins had highly elevated opercular rates (Fig. 1) and heart rates. The difference in activity was less pronounced in the later part of the period of yolk absorption (from day 30 on) because of the increasing ability of the alevin to support itself on its pectoral fins (Nortvedt 1987). In comparison, only minor differences in activity or opercular rate were found between ATR and FSR rainbow trout alevins, which in the period from hatching until swimup (day 28), swam a distance of 90.7 and 86.4 m, respectively. This is explained by less rolling and righting activity in the rainbow trout alevins because of the small yolk sac, a lower angle of the body axis to the bottom screen (which improved their balance), and a faster development rate (reflected by the shorter time from hatching until swimup in Atlantic salmon).

When the cover is taken off a hatching tray, FSR alevins are congregated in the corners and along the walls (Stuart 1953; Dill 1977; Hansen 1984; Nortvedt 1987). A few days after hatching, FSR Atlantic salmon and rainbow trout alevins scatter immediately upon the sudden increase in light intensity (Nortvedt 1987). ATR alevins, however, are always spread out on the substrate surface and do not react to sudden illumination.

## GROWTH

Under natural conditions, salmon cover their eggs with gravel. Babcock (1911) found that by covering salmon eggs with 15-18 cm of gravel in an incubator, mortality was reduced and the fry were stronger. Bams (1967) tested the effect of different incubation methods on swimming performance of fry and their vulnerability to predators. The main factor responsible for difference in performance was the size of the fry. Several authors have reported an improved yolk conversion efficiency and higher growth rate of salmonid alevins incubated in hatching substrates (Marr 1965; Bailey and Taylor 1974; Leon 1975; Eriksson and Westlund 1983; Hansen and Torrissen 1984; Hansen 1985; Hansen and Møller 1985 (Fig. 2).

Alevins utilize the energy required for growth and activity from the yolk (Heming and Buddington 1988). As a consequence of the increased catabolism due to hyperactivity (Hansen 1984), the FSR alevins convert less yolk to body tissue. Accordingly, FSR alevins have a reduced yolk conversion efficiency (YCE) (Hansen and Torrissen 1984; Hansen and Møller 1985). However, differences in yolk

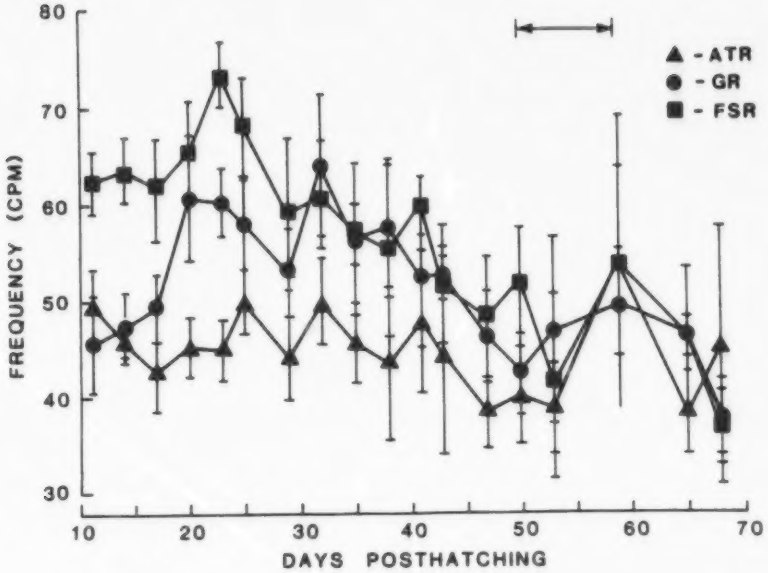


**Fig. 1.** Breathing (opercular) rate of Atlantic salmon reared on different incubation media, in relation to days posthatching, with 95% confidence intervals. Arrows indicate 50% swimup. CPM indicates count of opercular movements per minute.

▲ = Astroturf

● = Gravel reared

■ = Flat screen

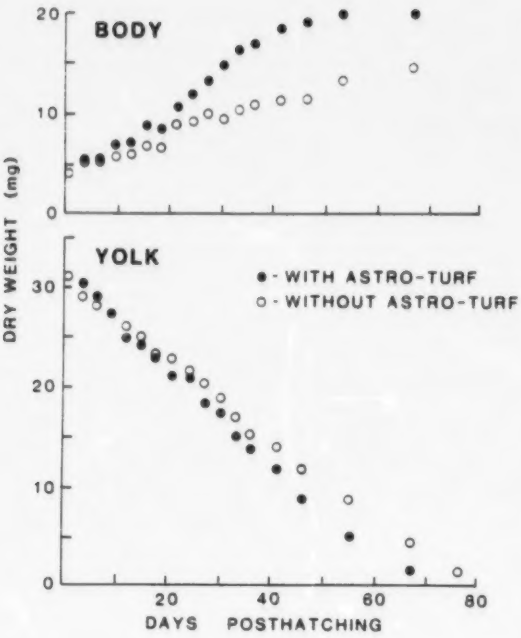


**Fig. 2. Changes in dry weight over time of body (upper panel) and yolk (lower panel) in the two incubation systems. Vertical lines indicate 95% confidence limits (from Hansen and Møller 1985).**

absorption rate can also contribute to the difference in growth because ATR alevins absorb relatively more of their yolk during periods of high yolk conversion efficiency than do FSR alevins (Hansen and Møller 1985).

## YOLK ABSORPTION RATE

Several authors have reported that yolk absorption rate (YAR) is nearly constant from hatching throughout the alevin stage (Brannon 1965; Peterson et al. 1983; Hansen and Møller 1985). However, this is not in agreement with observations made by Bams (1969), who found that YAR is a function of the quantity of yolk available and of temperature. According to Bams (1969) and Heming and Buddington (1988), it seems likely that the area of yolk syncytium covering the surface of the yolk determines the absorption rate at a given temperature. In addition, YAR is dependent on the metabolic activity of the yolk syncytium itself (Heming and Buddington 1988). As the YAR is directly or indirectly dependent on several environmental factors, one might expect that the high activity of the FSR alevins is compensated for by an increase in YAR. However, at a given temperature, YAR does not increase with increases in metabolism. Conversely, we have demonstrated that ATR alevins have a higher YAR than FSR alevins (Hansen 1984; Hansen and Torrissen 1984; Hansen and Møller 1985 (Fig. 2)). This supports the findings of Leon (1975) who used plastic saddles as an incubation substrate. He proposed that the lower YAR of the FSR alevins may be the result of oxygen depletion in their microenvironment. Hamor and Garside (1977) stated that low oxygen tension retarded yolk sac absorption in Atlantic salmon. However, it is unlikely that oxygen reached limiting concentrations in our incubation systems because of the high water exchange. The high activity of the FSR alevins might, however, reduce the YAR as other stressors have been shown to do (Table 1).

YAR normally increases with increasing temperature (Hamor and Garside 1977; Peterson et al. 1977; Gunnes 1979; Heming 1982). However, at very high temperatures there is a reduction in YAR (Table 1, 2).

In salmonids, the rate of yolk absorption shows a direct relation with egg size. Thus, all other factors being equal, alevins of a given salmonid species complete yolk absorption within a span of several days, despite large differences in egg size (Heming and Buddington 1988). Hansen and Torrissen (1984) found that YAR was significantly influenced by yolk weight at hatching. Thus, the higher YARs were found in groups with high yolk weights. This difference tends to reduce the variance in yolk weight during alevin growth and development. Under natural conditions, this may act to concentrate the time of fry emergence.

## YOLK SAC CONSTRICTIONS

Flat-bottomed incubation trays are known to induce yolk sac constrictions in different *Oncorhynchus* species and hybrids (Emadi 1973) and in Atlantic salmon (Leon 1975; Gunnes 1979; Hansen 1988). Yolk sac constrictions appear to be associated with the high swimming activity of alevins which creates a backward and lateral force on the yolk sac, causing it to elongate (Hansen and Møller

Table 1. Environmental stressors that have been shown to reduce the yolk absorption rate in salmonids.

| Stressor | Species | Reference |
|---|---|---|
| High temperature | *Salmo salar*<br>" | Hayes and Pelluet (1945)<br>Hansen et al. (Table 2, this paper) |
| PCB and DDT | *Oncorhynchus kisutch* | Halter and Johnson (1974) |
| Low $O_2$ conc. | *Salmo salar*<br>*Oncorhynchus nerka* | Hamor and Garside (1977)<br>Brannon (1965) |
| $NH_3$ | *Oncorhynchus gorbuscha* | Fedorov and Smirnova (1978) |
| Herbicides | *Salmo clarki*<br>*Salvelinus namaycush* | Woodward (1976)<br>" |
| Cadmium | *Salmo salar* | Peterson et al. (1983) |
| Low pH | *Salvelinus namaycush*<br>*Salmo salar* | Menendez (1976)<br>Skogheim and Rosseland (1984) |
| Cu | *Salmo salar* | McKim and Benoit (1971) |

Table 2. Yolk sac absorption rate in Atlantic salmon incubated at three different temperatures and on two different substrates, Astroturf (ATR) and flat screened hatching trays (FSR) from day 2 to 19.

| | YAR (mg yolk/day*degree) | | |
|---|---|---|---|
| Temperature, °C | 12 | 14 | 18 |
| ATR | 0.1005 | 0.0975 | 0.0944 |
| FSR | 0.0887 | 0.0912 | 0.0767 |

1985). As the sac gets slimmer and longer, the swimming activity probably forces the posterior portion sideways and a constriction is formed. Thus, the problem will be aggravated by factors that increase the activity of the alevins. High temperatures have been shown to induce yolk sac constrictions (Dumas 1966; Emadi 1973) as have high water velocities (Jochimsen and Bedell 1968; Emadi 1973). However, various substrates such as gravel (Emadi 1973), plastic saddles (Leon 1975) and Astroturf (Hansen and Møller 1985) are known to resolve the problem, probably by reducing the activity of the alevins.

If alevins with yolk sac constrictions are transferred to an incubation substrate at an early stage, the yolk posterior to constriction is absorbed normally. Therefore, yolk sac constrictions are not irreversible but rather a condition which is maintained and aggravated by high larval activity (Hansen 1988). At later stages of yolk sac constriction, the blood circulation through the yolk sac veins is impeded and the yolk coagulates and cannot be absorbed.

## REFERENCES

Babcock, J. P. 1911. Some experiments in the burial of salmon eggs suggesting a new method of hatching salmon and trout. Trans. Am. Fish. Soc. 40: 393-395.

Bailey, J. E., and S. G. Taylor. 1974. Salmon fry production in a gravel incubator hatchery, Auke Creek, Alaska, 1971-72. U.S. Dept. Commer. NOAA Tech. Memo. NMFS ABFL-3: 13 p.

Bams, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrants, as measured with swimming and predation tests. J. Fish. Res. Board Can. 24: 1117-1153.

Bams, R. A. 1969. Adaptations of sockeye salmon associated with incubation in stream gravels, p. 71-87. In T. G. Northcote (ed.) Symposium on salmon and trout in streams, 1968. H.R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

Brannon, E. L. 1965. The influence of physical factors on the development and weight of sockeye salmon embryos and alevins. Int. Pac. Salmon Fish. Comm. Prog. Rep. 12: 26 p.

Dill, P. A. 1977. Development of behaviour in alevins of Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*). Anim. Behav. 25: 116-121.

Dumas, R. F. 1966. Observations on yolk sac constriction in landlocked Atlantic salmon fry. Prog. Fish-Cult. 28: 73-75.

Edwards, D. J. 1978. Salmon and trout farming in Norway. Fishing News Books Ltd., Farnham, Surrey, England, 195 p.

Emadi, H. 1973. Yolk sac malformation in Pacific salmon in relation to substrate, temperature, and water velocity. J. Fish. Res. Board Can. 30: 1249-1250.

Eriksson, C., and G. Westlund. 1983. The impact on survival and growth of Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) by using incubators with artificial substrate. 1. Hatching and first summer. Laksforskningsinst. Meddelande 1983: 2.

Fedorov, K. Y., and Z. V. Smirnova. 1978. Dynamics of ammonia accumulation and its effect on the development of the pink salmon (*Oncorhynchus gorbuscha*), in closed circuit incubation systems. J. Ichthyol. 18: 288-295 (translated fromm Vaprosy ichtiologii).

Gunnes, K. 1979. Survival and development of Atlantic salmon eggs and fry at three different temperatures. Aquaculture 16: 211-218.

Halter, M. T., and H. E. Johnson. 1974. Acute toxicities of a polychlorinated biphenyl (PCB) and DDT alone and in combination to early life stages of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 31: 1543-1547.

Hamor, T., and E. T. Garside. 1977. Size relations and yolk utilization in embryonated ova and alevins of Atlantic salmon (*Salmo salar*) in various combinations of temperature and dissolved oxygen. Can. J. Zool. 55: 1892-1898.

Hansen, T. 1984. Klekking av laks (*Salmo salar*) i kunstgress. Hatching of Atlantic salmon (*S. salar*) in artificial turf. M.Sc. Thesis, Institute of Fisheries Biology, University of Bergen, 147 p. (in Norwegian).

Hansen, T. 1985. Artificial hatching substrate: effect on yolk absorption, mortality and growth during startfeeding of sea trout (*Salmo trutta*). Aquaculture 46: 275-285.

Hansen, T. 1988. The transfer of Atlantic salmon (*Salmo salar*) with constricted yolk sacs to an artificial hatching substrate. Aquaculture 69: 291-297.

Hansen, T., and D. Møller. 1985. Yolk absorption, yolk sac constrictions, mortality and growth during first feeding of Atlantic salmon (*Salmo salar*) incubated on Astro-turf. Can. J. Fish. Aquat. Sci. 42: 1073-1078.

Hansen, T., and O. Torrissen. 1984. Artificial hatching substrate in the mass rearing of larval Atlantic salmon (*Salmo salar*). Int. Counc. Explor. Sea C.M. 1984/F:22, 19 p.

Hayes, F. R., and D. Pelluet. 1945. The effect of temperature on the growth and efficiency of yolk conversion in the salmon embryo. Can. J. Res. 23: 7-15.

Heming, T. A. 1982. Effects of temperature on utilization of yolk by chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins. Can. J. Fish. Aquat. Sci. 39: 184-190.

Heming, T. A., and R. K. Buddington. 1988. Yolk absorption in embryonic and larval fishes, p. 407-466. In W. S. Hoar and D. J. Randall (ed.) Fish Physiology XI, the physiology of developing fish. Part A. Eggs and larvae. Academic Press, London.

Jochimsen, W., and G. Bedell. 1968. Malformed yolk sacs observed in chinook salmon fry during tests of barrel-type incubators. Prog. Fish-Cult. 30: 227-229.

Leon, K. A. 1975. Improved growth and survival of juvenile Atlantic salmon (*Salmo salar*) hatched in drums packed with a labyrinthine plastic substrate. Prog. Fish-Cult. 37: 158-163.

Marr, D. H. A. 1963. The influence of surface contour on the behaviour of trout alevins (*Salmo trutta* L.). Anim. Behav. 11: 412.

Marr, D. H. A. 1965. Factors affecting the growth of salmon alevins and their survival and growth during the fry stage. Yearbook Assoc. River Authorities 133-141.

McKim, J. M., and D. A. Benoit. 1971. Effects of long-term exposures to copper on survival, growth and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 28: 655-662.

Menendez, R. 1976. Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 118-123.

Nortvedt, R. 1987. Atferd hos yngel av laks og renbueørret. (Behaviour in Atlantic salmon and rainbow trout alevins). M.Sc. Thesis, Institute of Fisheries Biology, Univ. Bergen.

Peterson, R. H., J. L. Metcalfe, and S. Ray. 1983. Effects of cadmium on yolk utilization, growth, and survival of Atlantic salmon alevins and newly feeding fry. Arch. Environ. Contam. Toxicol. 12: 37-44.

Peterson, R. H., H. C. E. Spinney, and A. Sreedharan. 1977. Development of Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes. J. Fish. Res. Board Can. 34: 31-43.

Skogheim, O. K., and B. O. Rosseland. 1984. A comparative study on salmonid fish species in acid aluminium-rich water. I. Mortality of eggs and alevins. Rep. Inst. Freshwater Res., Drottningholm 61: 177-185.

Stuart, T. A. 1953. Spawning migration, reproduction and young stages of loch trout (*Salmo trutta* L.). Freshwater Salmon Fish. Res., Scottish Home Dept. 5: 39 p.

Woodward, D. F. 1976. Toxicity of the herbicides Dinoseb and Picloram to cutthroat (*Salmo clarki*) and lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 33: 1671-1676.

# THE EFFECT OF FISH DENSITY AND FEEDING REGIMES ON INDIVIDUAL GROWTH RATE AND MORTALITY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)[1]

Jens Chr. Holm, Terje Refstie[2] and Sigbjørn Bø[3*]
Austevoll Marine Aquaculture Station
Division of Aquaculture
Institute of Marine Research
N-5392 Storebø (Norway)

## ABSTRACT

Holm, J. C., T. Refstie, and S. Bø. 1990. The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*), p. 77. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Feeding frequency and fish density were varied in a 3 x 3 matrix rearing experiment with rainbow trout of initial size between 130 and 250 mm fork length. All fish groups were given similar daily rations. Initial densities were 107-219 $kg{\cdot}m^{-3}$, terminal 240-450 $kg{\cdot}m^{-3}$. The parameters of daily fork length increase, specific growth rate, and mortality were recorded. Mean individual growth rate, irrespective of feeding regime, was highest in the lowest density and lowest in the highest density. Growth rate increased with increased feed availability. High feed availability (high frequency of feeding) was especially important when densities were extremely high.

## RÉSUMÉ

Holm, J. C., T. Refstie, and S. Bø. 1990. The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*), p. 77. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Au cours d'une expérience sur l'élevage de truites arc-en-ciel dont la longueur à la fourche initiale varie de 130 à 250 mm, dans une matrice de 3 par 3, nous avons modifié la fréquence de l'alimentation et la densité du poisson. Tous les groupes de poissons ont reçu les mêmes rations quotidiennes. La densité initiale était de 107 à 219 kg au $m^3$ et la densité finale était de 240 à 450 kg au $m^3$. On a ensuite pris en note les paramètres de l'augmentation quotidienne de la longueur à la fourche, du taux de croissance spécifique et du taux de mortalité Si l'on fait abstraction du régime d'alimentation, le taux moyen de croissance individuelle était d'autant plus élevé que la densité était faible, et vice versa. Le taux de croissance augmente proportionnellement à la disponibilité accrue de nourriture. Il était particulièrement important que le poisson soit nourri très souvent aux moments où les densités étaient extrêmement élevées.

---

[1] Full article is published in Aquaculture 89: 225-232.
[2] The Agricultural Research Council of Norway, Institute of Aquaculture Research, N-6600 Sunndalsøra (Norway).
[3] Dept. of Fisheries Biology, University of Bergen, P. O. Box 1839 Nordnes, N-5024 Bergen (Norway).
[*] Present address: Skretting a.s., P. O. Box 319, N-4001 Stavanger (Norway).

# THE INFLUENCE OF LIGHT ON GROWTH AND SMOLTING OF ATLANTIC SALMON (*SALMO SALAR*): EFFECTS OF SPECTRAL COMPOSITION, INTENSITY AND PHOTOPERIOD

S. O. Stefansson, T. Hansen and G. L. Taranger[1]
Institute of Marine Research
Department of Aquaculture
Matre Aquaculture Station
N-5198 Matredal, Norway

[1]Dept. of Fisheries Biology, University of Bergen
P. O. Box 1839, N-5024 Bergen, Norway

## ABSTRACT

Stefansson, S. O., T. Hansen, and G. L. Taranger. 1990. The influence of light on growth and smolting of Atlantic salmon (*Salmo salar*): effects of spectral composition, intensity, and photoperiod, p. 79-84. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Juvenile Atlantic salmon (*Salmo salar*) were reared under different indoor light conditions to investigate the effects of spectral composition, light intensity, and photoperiod on growth and smolting. Of the three aspects of light, photoperiod was the most important factor for growth and smolting. Continuous light increased growth rate of juvenile Atlantic salmon during winter and spring, increasing the proportion of potential 1-yr smolts. The unchanging photoperiod, however, interfered with the parr-smolt transformation normally taking place under natural photoperiod. A combination of a continuous, low intensity background illumination and a superimposed natural photoperiod, termed a 'dual photoperiod', enhanced growth rate without seriously affecting the parr-smolt transformation. Within the range investigated in our studies, spectral composition and light intensity had little influence on growth and smolting of juvenile Atlantic salmon.

## RÉSUMÉ

Stefansson, S. O., T. Hansen, and G. L. Taranger. 1990. The influence of light on growth and smolting of Atlantic salmon (*Salmo salar*): effects of spectral composition, intensity, and photoperiod, p. 79-84. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Nous avons procédé à l'élevage du saumon de l'Atlantique juvénile (*Salmo salar*) en utilisant différents éclairages intérieurs, afin d'étudier les effets de la composition spectrale de la lumière, de l'intensité lumineuse et de la photopériode sur la croissance du saumon et le passage de l'état de tacon à l'état de smolt. Sur les trois aspects de l'éclairage, la photopériode est le facteur le plus déterminant dans la croissance et la smoltification. L'éclairage continuel a fait augmenter le taux de croissance du saumon de l'Atlantique juvénile durant l'hiver et le printemps, d'où une hausse dans la proportion de smolts éventuels d'un an. Par contre, la photopériode constante nuit à la transformation du tacon, qui a lieu normalement en photopériode naturelle. Une combinaison d'éclairage ambiant continu à faible intensité et de photopériode naturelle superposée, dite "photopériode double," a augmenté le taux de croissance sans incidence grave sur la smoltification. Dans le cadre de nos études, la composition spectrale de la lumière et l'intensité lumineuse avait peu d'influence sur la croissance et la transformation du saumon de l'Atlantique juvénile.

## INTRODUCTION

Naturally increasing photoperiod and temperature in the spring provide environmental cues influencing growth and synchronizing completion of parr-smolt transformation in Atlantic salmon (*Salmo salar*) (Wedemeyer et al. 1980; Hoar 1988). Parr-smolt transformation involves the development of hypoosmoregulatory ability as well as metabolic and behavioral changes that transform the dark, bottom-dwelling parr into a silvery, pelagic smolt, preadapted for a life in the ocean (Dickhoff and Sullivan 1987; McCormick and Saunders 1987; McCormick et al. 1987). In nature, these possibly unrelated processes are orchestrated, resulting in a complete parr-smolt transformation.

The Norwegian salmon farming industry relies entirely on farmed smolts. Much of this production takes place indoors. Hence, critical steps of the parr-smolt transformation take place under artificial light conditions which may differ from natural in respect to spectral composition, intensity, and photoperiod. To ensure normal completion of smolting, it is important to gain better understanding of the influence of light on parr-smolt transformation and growth. This will also lead to greater understanding of the processes that together constitute the parr-smolt transformation.

When smolting salmonids are reared indoors and deprived of their natural environmental cues, several problems may arise. Photoperiods other than a simulated natural one are known to affect growth rate and parr-smolt transformation (Clarke et al. 1978, 1981; Lundqvist 1980; Brauer 1982; Saunders et al. 1985; McCormick et al. 1987; Stefansson et al. 1989). This review of our work will focus on the effects of spectral composition, light intensity, and photoperiod on growth and parr-smolt transformation in Atlantic salmon.

## EFFECTS OF SPECTRAL COMPOSITION AND LIGHT INTENSITY ON GROWTH AND SMOLTING

Stefansson and Hansen (1989a) concluded that growth rate of juvenile Atlantic salmon was not influenced by differences in spectral composition within the visible spectrum of the light. Further, all changes normally associated with parr-smolt transformation were completed irrespective of spectral composition. All groups had low condition coefficient and showed 100% survival for 96 h in 40 o/oo salinity in early May. The morphological signs of parr-smolt transformation (loose, silvery scales, dark fin margins, and absence of parr marks) were present in all groups. Growth rate during 6 mo of rearing in cages in seawater was similar in all groups, suggesting that the metabolic changes associated with the parr-smolt transformation were completed in all groups.

In a study of the effects of photoperiod and light intensity during first feeding of Atlantic salmon (Stefansson et al. 1990), no consistent differences in growth rate were found between the groups subjected to different light intensities, ranging from 27-1400 lux, suggesting that light intensity has little effect on first feeding of Atlantic salmon. These results agree with those reported for chinook salmon (*Oncorhynchus tshawytscha*, Eisler 1957) and rainbow trout (*O. mykiss*, Kwain 1975). In contrast, changes in photoperiod had a significant effect on growth and survival of first feeding fry. Growth rate and survival were substantially lower in the group on simulated natural photoperiod (increasing from LD10$^{3}/_{4}$:13$^{1}/_{4}$ to LD20:4) than in the groups on continuous light with significantly higher mortalities during the first 3 wk of feeding. These results suggest that an initial daylength less than 12 h may be too short to allow successful first feeding of Atlantic salmon. Growth rate under a dual light regime, consisting of a continuous low intensity background illumination and a superimposed high intensity simulated natural photoperiod (Stefansson and Hansen 1989b), was the same as under continuous light. Therefore, within wide limits, light intensity does not influence growth during first feeding.

In a second experiment, potential 1+ Atlantic salmon smolts were reared under continuous light from first feeding in March until the start of the experiment on 4 November 1987, when they were subjected to a simulated natural photoperiod for 60°N at either 27 lux (LI), 335 lux (MI), or 715 lux (HI). Water temperature decreased from 7°C in November to 2°C in mid-December, after which the water was heated, raising temperatures to 11 ± 1°C. The fish were fed a commercial dry diet with daily rations calculated according to temperature and fish size. Seawater exposure tests to assess hypoosmoregulatory ability were performed on 9 February (32.5 o/oo, 24 h), 17 March (35.0 o/oo, 24 h), and 25 April (37.5 o/oo, 96 h). A control group was retained in fresh water. On 3 May, each group was identified by distinctive fin clipping, acclimated to seawater, and transferred to six 3-m cylindrical tanks under ambient conditions of light, temperature, and salinity. On 3 October, the fish were transferred to a 5-m tank supplied with seawater from the same source.

Different light intensities had little effect on growth or parr-smolt transformation in Atlantic salmon. There were no significant differences in mean length among the groups (Table 1). Specific growth rate (calculated as $SGR = (e^g-1)100\%$, where $g = \ln(l2) - \ln(l1)(t2-t1)^{-1}$), where l1 and l2 are mean lengths at times t1 and t2) was similar in all groups throughout the freshwater period. The condition coefficient ($cc = 100\ w/l^3$) increased in all groups from mid-December to the end of January following the increase in temperature, and decreased until the termination of the experiment, characteristic of fish undergoing parr-smolt transformation (Farmer et al. 1978). There were no significant differences in mean plasma chloride levels among the groups (Table 2). All groups showed between 90 and 100% survival during the tolerance test in April. There were no significant differences in mean length among the groups after 5 mo of rearing in seawater (Table 1). The mortality was <1% during this period and the similar growth rates of all groups do not indicate any differences in smolt status.

Table 1. Specific growth rate (% $day^{-1}$) and mean length (cm) of juvenile salmon reared under different light intensities. Mean lengths are at time of transfer to seawater (May 3) and after 7 mo of rearing in seawater (15 December). % maturation is as postsmolts in their first winter in seawater. LI = 27 lux, MI = 335 lux, and HI = 715 lux.

| | Period | | | | | | | Length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | Nov. 4-Dec. 16 | Dec. 17-Jan. 28 | Jan. 29-Mar. 1 | Mar. 2-Apr. 4 | Apr. 5-May 3 | May 4-Oct. 3 | Oct. 4-Dec. 15 | May 3 | Dec. 15 | Maturation (%) |
| LI | 0.12 | 0.29 | 0.26 | 0.31 | 0.25 | 0.31 | 0.28 | 20.5 | 40.4 | 12.7 |
| MI | 0.11 | 0.29 | 0.27 | 0.31 | 0.22 | 0.31 | 0.27 | 20.3 | 39.8 | 12.9 |
| HI | 0.09 | 0.30 | 0.28 | 0.30 | 0.29 | 0.31 | 0.27 | 20.5 | 40.0 | 16.4 |

Table 2. Results from the seawater exposure tests. Performance is presented as mean plasma chloride levels (mM) and standard error of mean (sem). Salinities and exposure times were 32.5 o/oo, 24 h for 9 Feb., 35 o/oo, 24 h for 17 Mar., and 37.5 o/oo, 96 h for 25 April. FW is combined from freshwater control fish from all three groups. These were not significantly different and hence were combined to form a freshwater control group.

| | Date | | | |
|---|---|---|---|---|
| Group | Feb. 9 | Mar. 17 | Apr. 25 | % survival |
| LI | 148.3 (3.6) | 141.5 (1.6) | 146.2 (4.8) | 91.7 |
| MI | 148.4 (2.4) | 140.7 (4.6) | 140.7 (1.1) | 100.0 |
| HI | 146.7 (2.3) | 140.7 (1.7) | 138.3 (0.8) | 100.0 |
| FW | 126.7 (1.1) | 133.1 (2.1) | | |

## EFFECTS OF PHOTOPERIOD ON GROWTH AND SMOLTING

Stefansson et al. (1989) reported that three unchanging photoperiods (LD24:0), LD16:8, or LD8:16) significantly affected growth and smolting. Continuous light had a growth promoting effect in this experiment, and a higher percentage of the population recruited into the upper mode under LD24:0. Although large and healthy, the upper mode fish did not develop the morphological characters of true smolts. This finding was in agreement with those reported by Saunders et al. (1985) and McCormick et al. (1987). This growth promoting effect of extended daylength and continuous light is well documented for salmonids in fresh water (Pyle 1969; Clarke et al. 1978, 1981; Lundqvist 1980; Saunders et al. 1985; McCormick et al. 1987). Growth rate was highest under LD24:0 except between 9 and 27 May, when the fish under LD8:16 showed the highest growth rate. There was no reduction in condition coefficient under any photoperiod in late winter or early spring, suggesting that important aspects of parr-smolt transformation were not completed. However, an increase in plasma cortisol towards the end of the experiment indicated that the fish were undergoing certain parts of the parr-smolt transformation (Specker and Schreck 1982; Thorpe et al. 1987). Taken together, the high condition coefficient and lack of morphological smolt characteristics indicate that the parr-smolt transformation was not completed under any of the unchanging photoperiods.

In a recent experiment, potential 1+ smolts of Atlantic salmon which had been reared under natural temperature and light conditions were transferred to indoor tanks and subjected to different photoperiods (S. O. Stefansson, unpubl. data). Continuous light (LD 24:0) and dual photoperiod (LDD, described above) significantly enhanced growth rate in this study. In contrast with the experiment reported above (Stefansson et al. 1989), there was also a significant reduction in condition coefficient and increase in hypoosmoregulatory ability in the group on LD24:0. An increase in hypoosmoregulatory ability and reduction in condition coefficient was also reported by Duston and Saunders (1990) following an abrupt increase in daylength to LD16:8. However, the LD24:0 group had poorer hypoosmoregulatory ability than the LDN and LDD groups at the normal time of transfer to seawater (early May). This indicates that the fish from LD24:0 did not achieve true smolt status, or that they completed their parr-smolt transformation earlier and were subsequently loosing some smolt characteristics (Hoar 1988).

The higher growth rate of the LDD and LDN groups in seawater compared with the fish from continuous light suggests that parts of the parr-smolt transformation may have been incomplete under the LD24:0 or that the time of seawater transfer was not correct for this group. Taken together, the low coefficient of condition, the high hypoosmoregulatory ability and the high growth rate in seawater suggest that the fish from the LDD were fully developed smolts. The high overall growth rate in seawater in this experiment suggests that although fish from LD24:0 may be inferior to fish from LDN regarding some aspects of parr-smolt transformation, or are transferred past their optimum time, they may still survive and grow well in seawater.

Results on early maturation as postsmolts and grilse indicate that photoperiod treatment in fresh water may influence the maturation cycle in seawater. The higher incidence of mature postsmolts may reflect the high growth rate and large smolt size of the groups previously exposed to continuous light, whereas the higher percentage of grilse in the control group may reflect the higher growth rate the first autumn and early winter in seawater.

## GENERAL DISCUSSION

Of the different aspects of light investigated, photoperiod is clearly the most important for growth and parr-smolt transformation in Atlantic salmon. Within wide limits, the spectral composition and intensity of the light have little effect on growth and smolting of juvenile salmon.

Extended photoperoid and continuous light improve growth of juvenile Atlantic salmon in fresh water (Saunders et al. 1985, 1989; McCormick et al. 1987; Saunders and Henderson 1988; Stefansson et al. 1989; Saunders and Harmon 1990). The improved growth may be the result of photoperiod advancing endogenous rhythms that influence growth rate and timing of parr-smolt transformation (Eriksson and Lundqvist 1982; Clarke et al. 1985). The time-limited effect of continuous light on growth may be the result of photoperiod acting through phase adjustment of endogenous rhythms (Wagner 1974; Clarke et al. 1978), or through photostimulated release of growth hormone for a limited period (Marchant and Peter 1986).

The completion of parr-smolt transformation has traditionally been regarded as depending on an increase in photoperiod (Hoar 1976, 1988; Wedemeyer et al. 1980). Experiments indicate that continuous light does not allow for the necessary orchestration of the different processes which result in satisfactory smolt quality (Saunders et al. 1985; McCormick et al. 1987; Stefansson et al. 1989). Recent findings, however, seem to indicate that under certain conditions, with fish of a certain size, continuous light may be sufficient to ensure the development of important smolt characteristics. These somewhat contradictory findings may find their explanation in causes other than photoperiod. The pre-treatment history (photoperiod and temperature), size distribution of the fish, and experimental temperatures were different in the two experiments described above. An abrupt increase in photoperiod to continuous light in the winter may be sufficient to complete the final stages of smolting if the fish had reached an appropriate size and physiological-biochemical state to respond correctly. The combination of a continuous background illumination and a simulated natural photoperiod (dual photoperiod) holds promise as a way to couple the high growth rate of continuous light and the good smolt quality and normal timing of smolting associated with natural photoperiod.

## ACKNOWLEDGMENTS

We wish to thank Drs. R. L. Saunders and J. Duston for helpful suggestions during preparation of the manuscript.

## REFERENCES

Brauer, E. P. 1982. The photoperiod control of coho salmon smoltification. Aquaculture 28: 105-111.

Clarke, W. C., H. Lundqvist, and L.-O. Eriksson. 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol. 26: 29-35.

Clarke, W. C., J. E. Shelbourn, and J. R. Brett. 1978. Growth and adaptation to seawater in 'underyearling' sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon subjected to regimes of constant or changing temperature and daylength. Can. J. Zool. 56: 2413-2421.

Clarke, W. C., J. E. Shelbourn, and J. R. Brett. 1981. Effect of artificial photoperiod cycles, temperature and salinity on growth and smolting in underyearling coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*) and sockeye (*O. nerka*) salmon. Aquaculture 22: 105-116.

Dickhoff, W. W., and C. V. Sullivan. 1987. Involvement of the thyroid gland in smoltification, with special reference to the metabolic and developmental processes. Am. Fish. Soc. Symp. 1: 197-210.

Duston, J., and R. L. Saunders. 1990. The entrainment role of photoperiod on hypoosmoregulatory and growth related aspects of smolting in Atlantic salmon (*Salmo salar*). Can. J. Zool. 68: 707-715.

Eisler, R. 1957. The influence of light on the early growth of chinook salmon. Growth 21: 197-203.

Eriksson, L.-O., and H. Lundqvist. 1982. Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). Aquaculture 28: 113-121.

Farmer, G. J., J. A. Ritter, and D. Ashfield. 1978. Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 25: 93-100.

Hoar, W. S. 1976. Smolt transformation: evolution, behaviour and physiology. J. Fish. Res. Board Can. 33: 1234-1252.

Hoar, W. S. 1988. The physiology of smolting salmonids, p. 275-343. In W. S. Hoar and D. J. Randall (ed.) Fish physiology, Vol. 11B, Academic Press, New York.

Kwain, W.-H. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature. J. Fish. Res. Board Can. 32: 397-402.

Lundqvist, H. 1980. Influence of photoperiod on growth in Baltic salmon parr (*Salmo salar* L.) with special reference to the effect of precocious sexual maturation. Can. J. Zool. 58: 940-944.

Marchant, T. A., and R. E. Peter. 1986. Seasonal variations in body growth rates and circulating levels of growth hormone in the gold fish, *Carassius auratus*. J. Exp. Zool. 237: 231-239.

McCormick, S. D., and R. L. Saunders. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. Am. Fish. Soc. Symp. 1: 211-229.

McCormick, S. D., R. L. Saunders, E. B. Henderson, and P. R. Harmon. 1987. Photoperiod control of parr-smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill $Na^+,K^+$-ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquat. Sci. 44: 1462-1468.

Pyle, E. A. 1969. The effect of constant light or constant darkness on the growth and sexual maturity of brook trout. Cortland Hatchery Rep. 26(1967). N.Y. Conserv. Dep., Fish. Res. Bull. 31: 13-19.

Saunders, R. L., and P. R. Harmon. 1990. Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter-spring. Trans. Am. Fish. Soc. 119 (in press).

Saunders, R. L., and E. B. Henderson. 1988. Effect of constant day length on sexual maturation and growth of Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 45: 60-64.

Saunders, R. L., E. B. Henderson, and P. R. Harmon. 1985. Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture 45: 55-66.

Saunders, R. L., J. L. Specker, and M. P. Komourdjian. 1989. Effects of photoperiod on growth and smolting in juvenile Atlantic salmon (*Salmo salar*). Aquaculture 82: 103-117.

Specker, J. L., and C. B. Schreck. 1982. Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 46: 53-58.

Stefansson, S. O., and T. Hansen. 1989a. The effect of spectral composition on growth and smoltification in Atlantic salmon (*Salmo salar*) and subsequent growth in sea cages. Aquaculture 82: 155-162.

Stefansson, S. O., and T. Hansen. 1989b. Effects of tank colour on growth and smoltification of Atlantic salmon (*Salmo salar* L.). Aquaculture 81: 379-386.

Stefansson, S. O., G. Nævdal, and T. Hansen. 1989. The influence of three unchanging photoperiods on growth and parr-smolt transformation in Atlantic salmon, *Salmo salar* L. J. Fish Biol. 35: 237-247.

Stefansson, S. O., R. Nortvedt, T. Hansen, and G. L. Taranger. 1990. First feeding of Atlantic salmon (*Salmo salar*) under different photoperiods and light intensities. Aquacult. Fish. Manage. (in press).

Thorpe, J. E., M. G. McConway, M. S. Miles, and J. S. Muir. 1987. Diel and seasonal changes in resting plasma cortisol levels in juvenile Atlantic salmon, *Salmo salar* L. Gen. Comp. Endocrinol. 65: 19-22.

Wagner, H. A. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219-234.

Wedemeyer, G. A., R. L. Saunders, and W. C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1-14.

# SEA LICE INFESTATION ON FARMED SALMON: POSSIBLE USE OF CLEANER-FISH AS AN ALTERNATIVE METHOD FOR DE-LOUSING

Åsmund Bjordal
Institute of Fisheries Technology Research
P. O. Box 1964
5024 Bergen, Norway

## ABSTRACT

Bjordal, Å. 1990. Sea lice infestation on farmed salmon: possible use of cleaner-fish as an alternative method for de-lousing, p. 85-89 . In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Sea lice infestation is a major problem in intensive cage culture of Atlantic salmon and the current chemical treatment has several negative aspects. This paper describes experiments utilizing cleaning symbiosis between wrasses and salmon as an alternative method for de-lousing salmon in sea cages. Promising results indicate that two or three different wrasse species might be used as cleaner-fish for effective parasite control in commercial salmon farming.

## RÉSUMÉ

Bjordal, Å. 1990. Sea lice infestation on farmed salmon: possible use of cleaner-fish as an alternative method for de-lousing, p. 85-89. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'infestation par des parasites marins est un problème majeur dans la culture intensive en cages du saumon de l'Atlantique, et le traitement chimique actuellement appliqué comporte plusieurs inconvénients. Ce rapport décrit les expériences de symbiose d'épuration labre-saumon comme solution de rechange pour débarrasser le saumon en cages marines de ses parasites. Des résultats encourageants indiquent que deux ou trois espèces différentes de labres peuvent être utilisées comme agents d'épuration pour lutter efficacement contre les parasites dans la salmoniculture commerciale.

## INTRODUCTION

Epidemic infestation of sea lice (*Lepeophtheirus salmonis*) is a serious problem in intensive culture of Atlantic salmon (*Salmo salar*). The common solution to this problem is treatment with the chemicals Neguvon (Brandal and Egidius 1979) or Nuvan, a method that effectively de-louses the salmon. However, this is a costly and laborious procedure which, on many farms, has to be repeated several times a year. The active ingredient in Neguvon/Nuvan (dichlorvos) can be toxic to marine life in the vicinity of the farm (Egidius and Møster 1987) and can be a health risk to farm workers if not properly used.

Lice infestation and the associated chemical treatment are major stressors to salmon. Furevik et al. (1988) showed that there is significantly decreased leaping and increased rolling activity of salmon after de-lousing. High rolling activity (surfacing) is interpreted as a secondary stress response, the primary response being release of swimbladder gas, followed by rolling to ingest air for buoyancy compensation. Other indicators of stress, particularly increased heart rate and blood cortisol levels, have been correlated with the de-lousing process (Bjordal et al. 1988).

Efforts have been made to find simpler and less harmful ways of solving the sea lice problem, such as capture of lice based on chemo- or phototaxis or repelling the lice by sound or electric shock. So far, none of these techniques has proven to be effective. Shading of cages to 70 or 40% of ambient light level has been shown to give a slightly reduced lice infestation (Huse et al., in prep.)

Promising results are, however, obtained using cleaner-fish for de-lousing of farmed salmon. Bjordal (1988) found that several wrasse species were functional cleaners using Atlantic salmon as host species. Goldsinny (*Ctenolabrus rupestris*) and rock cook (*Centrolabrus exoletus*) were the most active cleaners, while female cuckoo wrasse (*Labrus ossifagus*) had a more modest cleaning behavior. This paper describes further experiments in sea cages on the utilization of wrasses for de-lousing of salmon.

## METHODS AND MATERIALS

Wrasses used in these experiments were caught locally by fyke nets, pots or beach seines.

### SMALL CAGE EXPERIMENTS

The experiments were conducted at the Austevoll Marine Aquaculture Station south of Bergen, Norway. Eight (5 x 5 m by 4 m deep) cages with 10 x 10 mm square mesh were used. At the start of the experiment (Aug. 17, 1988), 220 smolts with a mean weight of 84 g were stocked in each cage. The smolts had no visible lice infestation. Two cages were used as control groups, while the others were stocked with different species and numbers of wrasses. The smolt was fed to satiation with dry pelleted feed. Dead wrasses were replaced, except for rock cook, which was not available beyond those originally stocked in the cages. Lice infestation and salmon and wrasse mortalities were recorded on Aug. 30, Sept. 29 and Nov. 1, 1988. On the last two dates, samples of all smolt groups were measured and weighed. The lice infestation level was recorded in the following categories:

| Category | Number of lice per smolt |
|---|---|
| 1 | 0 |
| 2 | 1-5 |
| 3 | 6-10 |
| 4 | 11-20 |
| 5 | >20 |

De-lousing (with Nuvan) was done when necessary, according to the judgment of the farm manager.

### FULL-SCALE TRIALS

This experiment was done at a commercial fish farm, Austefjordlaks A/S - at the island of Sotra, west of Bergen. The experiment included three adjacent standard size cages (12 x 12x 6 m), with 20,000, 26,000 and 30,000 smolts. On Sept. 12, 1988, 500 goldsinny and 100 rock cook were placed in the middle cage with 26,000 smolts, while the other two cages were used as control groups. The smolts which had been transferred to sea cages in June were all de-loused with Nuvan 2 d prior to the start of the experiment. The only data recorded were the dates of de-lousing. Time of de-lousing was decided by the farm manager. The experiment was terminated on Nov. 21, 1988, when smolts were pooled into larger cages.

## RESULTS

## SMALL CAGE EXPERIMENTS

### Lice infestation

Lice infestation level, expressed as the average category value, is given in Table 1. A heavy lice infestation occurred shortly after the start of the experiment. Control group I and both groups with cuckoo wrasse had to be de-loused with Nuvan after 2 wk. Control group II suffered from high mortality and was significantly weakened due to the lice infestation and was therefore taken out of the experiment. Control group I needed two more chemical de-lousings, and a new control group II required one de-lousing by early November. During the same period, the lice infestation level was constantly low to moderate in the goldsinny and/or rock cook groups. Also, the cuckoo wrasse groups needed no further de-lousing by chemicals.

### Mortalities of smolts and wrasses

The mortalities of smolts and wrasses are given in Table 2. Smolt mortality was very high in both control groups and also in the cuckoo wrasse groups (57-70%), while the smolt mortalities in the remaining wrasse groups were low to moderate (0-16%). Wrasse mortality was high in the 50 rock cook group, while the mortalities in the other groups were low.

### Growth of smolts

Growth data for the different smolt groups (except control group II) are given in Table 3. Control group I showed the poorest growth, the cuckoo wrasse groups intermediate, while the highest average weights were noted in the 50 goldsinny, 50 rock cook and 15 goldsinny/15 rock cook groups.

## FULL-SCALE TRIALS

During the experimental period, only a few smolts were observed with lice infestation in the wrasse cage. The control group with 20,000 smolts suffered repeated lice attacks, and de-lousing with Nuvan was done three times during the period (Sept. 19, Oct. 10 and Nov. 14, 1988), while the second control group was de-loused once (Nov. 14).

Table 1. Small cage experiment, 1988. Lice infestation levels (according to category values given in text) and de-lousings (DL).

| Date | C-I | C-II* | 25CW | 50CW | 25G | 50G | 50RC | 15/15 | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 17 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | Experiment start |
| Aug. 30 | 4.80 | 5.00 | 4.70 | 4.50 | 2.30 | 1.90 | 1.20 | 1.20 | |
| Aug. 31 | DL | | - | - | - | - | - | - | |
| Sept. 1 | - | | DL | DL | - | - | - | - | |
| Sept. 29 | 3.66 | | 2.26 | 1.94 | 1.22 | 1.06 | 1.02 | 1.04 | |
| Oct. 5 | DL | 1.00 | - | - | - | - | - | - | |
| Nov. 1 | 3.52 | 3.28 | 1.68 | 1.28 | 1.36 | 1.30 | 1.06 | 1.02 | |
| Nov. 4 | DL | DL | - | - | - | - | - | - | |

C = control, CW = cuckoo wrasse, G = goldsinny, RC = rock cook, 15/15 = 15G + 15RC

*Due to high mortality caused by severe lice infestation, the C-II control group was taken out of the experiment on Aug. 30. A new group of 200 smolts was stocked in the cage on Oct. 5.

Table 2. Small cage experiments, 1988. Mortality of smolts and wrasses given as total number of original fish remaining. S = smolts, W = wrasses (explanation of other abbreviations, see Table 1). %M = percent mortality during the experiment.

| | C-I | | C-II | | 25CW | | 50CW | | 25G | | 50G | | 50RC | | 15/15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | S | W | S | W | S | W | S | W | S | W | S | W | S | W | S | W |
| Aug. 17 | 220 | - | 220 | - | 220 | 25 | 220 | 50 | 220 | 25 | 220 | 50 | 220 | 50 | 220 | 30 |
| Aug. 30 | 217 | - | 138 | - | 213 | 24 | 211 | 47 | 205 | 25 | 213 | 50 | 220 | 26 | 219 | 29[*] |
| Sep. 19 | 79 | - | - | - | 68 | 23 | 105 | 46 | 185 | 25 | 207 | 49 | 220 | 18 | 217 | 29 |
| Nov. 1 | 75 | - | - | - | 67 | 23 | 105 | 46 | 184 | 24 | 206 | 48 | 220 | 5 | 217 | 29 |
| % M | 66 | | - | | 70 | 8 | 52 | 8 | 16 | 4 | 6 | 4 | 0 | 90 | 1 | 3 |

[*] One dead rock cook.

Table 3. Small cage experiment, 1988. Growth data of the different smolt groups given as mean weights with the range in parentheses. n = 50 for all groups. Weight at start of experiment (Aug. 17): 84 g, range 35-155 g, n = 138 of total 1760 smolts. See Table 1 for explanation of abbreviations.

| Date | C-I | 25CW | 50CW | 25G | 50G | 50RC | 15/15 |
|---|---|---|---|---|---|---|---|
| Sept. 29 | 125<br>(70-210) | 124<br>(65-190) | 131<br>(75-200) | 158<br>(85-270) | 154<br>(90-245) | 152<br>(70-280) | 157<br>(80-275) |
| Nov. 1 | 220<br>(110-240) | 252<br>(110-460) | 253<br>(90-420) | 276<br>(100-480) | 290<br>(130-470) | 292<br>(80-520) | 297<br>(140-490) |
| % increase | 162 | 200 | 201 | 229 | 245 | 248 | 254 |

## DISCUSSION

Based on earlier findings on cleaning symbiosis between wrasses and lice-infested salmon (Bjordal 1988), the experiments described in this paper clearly indicate that wrasses can be used as cleaner-fish for salmon in sea cage culture. Even under intensive lice attacks, smolts in cages that were stocked with goldsinny and/or rock cook had only slight lice infestation. The cuckoo wrasse also showed good cleaning abilities but with somewhat lower effectiveness compared with the other two species. Smolt mortality showed a fairly good correlation with lice infestation, with the highest mortalities in the control and cuckoo wrasse groups. However, these results were obtained in small cage experiments, and may not apply to a full-scale operation. Wrasse mortality was high only in the cage with 50 rock cook (90%). Bjordal (1988) observed that rock cook generally showed more aggressive behavior than goldsinny, and intraspecific aggression combined with relatively high density might explain the high mortality. The low mortality of rock cook in the combined group with goldsinny support this hypothesis, although the existing material does not allow any conclusion on this point.

The smolt growth data should also be interpreted with care. Although the growth of the goldsinny and rock cook groups was superior to the control and cuckoo wrasse groups, the difference in growth might have been caused by other factors such as different densities due to different mortality rates or an effect of intestinal parasites that were found in some of the groups. However, there is reason to expect better growth of fish with low lice infestation compared with fish that are stressed by repeated lice attacks followed by chemical treatment.

The experiment conducted at the commercial fish farm was the first trial of this method in a full-scale operation. Promising results were obtained at a much lower wrasse-to-smolt ratio (1:50) than in the small cage experiments. The group with 50 rock cook, however, ended up at a somewhat similar ratio (5 wrasse to 220 smolts), as there was no replacement of dead wrasse in this group. Effective cleaning might, therefore, be obtained at a 1:50 wrasse/smolt ratio. However, further investigations are needed to establish optimal ratios between wrasse and smolts.

## REFERENCES

Bjordal, Å. 1988. Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. Int. Counc. Explor Sea C.M. 1988, F:17.

Bjordal, Å., A. Fernø, D. Furevik, and I. Huse. 1988. Effects on salmon (*Salmo salar*) from different operational procedures in fish farming. Int. Counc. Explor. Sea C.M. 1988, F:16.

Brandal, P. O., and E. Egidius. 1979. Treatment of salmon lice (*Lepeophtheirus salmonis* Krøyer, 1938) with Neguvon®-description of method and equipment. Aquaculture 18: 183-188.

Egidius, E., and B. Møster. 1987. Effect of Neguvon® and Nuvan® treatment on crabs (*Cancer pagurus, C. maenas*), lobster (*Homarus gammarus*) and blue mussel (*Mytilus edulis*). Aquaculture 60: 165-168.

Furevik, D. M., I. Huse, Å Bjordal, and A. Fernø. 1988. Surface activity of Atlantic salmon (*Salmo salar*) in net pens. Int. Counc. Explor. Sea C.M. 1988, F:19.

# ACTIVITIES IN NUTRITIONAL RESEARCH AT THE INSTITUTE OF MARINE RESEARCH, DIVISION OF AQUACULTURE

Ole J. Torrissen
Institute of Marine Research
Matre Aquaculture Research Station
N-5198 Matredal, Norway

and

Igegjerd Opstad
Institute of Marine Research
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

## ABSTRACT

Torrissen, O. J., and I. Opstad. 1990. Activities in nutritional research at the Institute of Marine Research, Division of Aquaculture, p. 91-97. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The Institute of Marine Research, Bergen is involved in a wide spectrum of nutritional studies on salmonids and marine fishes, alone or in cooperation with other research institutions and industry. This paper briefly describes the progress done in the field of salmonid flesh pigmentation in relation to diet concentration of astaxanthin and in startfeeding diets of marine fish larvae. Flesh pigmentation of salmonids increases by increasing dietary astaxanthin or canthaxanthin level, but the retention rates decrease. This is due partly to a decrease in digestibility, but some of the pigments also seem to be metabolized. Formulated diets based on roe as the main feed ingredient gave growth and prolonged survival in cod and halibut; in plaice, a few larvae reached metamorphosis. Rotifers enriched with cod roe meal have so far given the highest growth and survival rate.

## RÉSUMÉ

Torrissen, O. J., and I. Opstad. 1990. Activities in nutritional research at the Institute of Marine Research, Division of Aquaculture, p. 91-97. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Le Institute of Marine Research de Bergen effectue toute une gamme d'études sur l'alimentation des salmonidés et des poissons de mer, études qu'il mène seul ou en collaboration avec d'autres établissements de recherche et l'industrie. Ce document décrit brièvement les progrès accomplis dans le domaine de la pigmentation de la chair des salmonidés en relation avec la concentration de l'asthaxanthine dans l'alimentation et dans l'alimentation de départ des larves de poisson de mer. La pigmentation de la chair des salmonidés devient plus prononcée lorsque l'on augmente le niveau d'asthaxanthine ou de canthaxanthine dans l'alimentation, mais les taux de rétention diminuent. Ceci est en partie dû à une diminution de la digestibilité, quoique certains des pigments semblent aussi se métaboliser. Les régimes préparés à base de rogue comme ingrédient principal ont favorisé la croissance et un taux de longévité prolongé chez la morue et le flétan; dans le cas de la plie, quelques larves se sont métamorphosées. L'alimentation à base de rotifères enrichis à la farine de rogue de morue a jusqu'à maintenant assuré le taux de croissance et de survie le plus élevé.

## INTRODUCTION

The 642 hatcheries and 791 salmon farms in Norway produced 114,866 tons of Atlantic salmon in 1989 (Anon. 1990). This represents a total feed consumption of about 130,000-150,000 tons of dry feed, giving an annual feed cost in the scale of $175 million US. It is assumed that the feed cost accounts for about 40% of the total production costs. In addition to representing the foundation for growth, feed and feeding are important for fish health and the quality of the product presented to the market.

In relation to the importance of nutrition in aquaculture, the activities in nutritional research at the Institute of Marine Research, Division of Aquaculture, seem unpretentious. For 1989, the Division was conducting six projects with a focus on nutrition:

1. Isozyme patterns of trypsin, feed utilization and growth in Atlantic salmon;
2. Biological function of astaxanthin in Atlantic salmon;
3. Pigmentation of Atlantic salmon. Dose response effects of supplementing dietary astaxanthin;
4. Live feeds for marine fish larvae, laboratory routines;
5. Feeding, growth, slaughter weight, and maturation of cod in intensive farming;
6. Dry starter diets for marine fish larvae.

However, this list does not give a complete picture of the activity as a large part of our activities in fish nutrition are in cooperation with other institutions, i.e., Institute of Nutrition, University of Bergen, the Herring Meal and Herring Oil Research Institute (SSF), and the private industry. Projects where cooperative institutions are project coordinators are not included in the above list, but include projects in:

1. Vitamin and mineral research;
2. Effect of fat quality on growth, health and meat quality;
3. Hydrolyzed fish and fish meal as constituents in starter diets for salmonids and marine fish species.

It is not feasible to give a comprehensive picture of our activity in nutrition research here; this presentation will, therefore, concentrate on the pigmentation of salmonids and the efforts on developing a dry diet for marine fish larvae.

## EFFECT OF DIETARY CAROTENOID LEVEL ON SALMONID FLESH PIGMENTATION

The strategy for achieving satisfactory pigmentation of Atlantic salmon and rainbow trout has gone through large changes during the last 20 yr. In Norway, shrimp waste from the shrimp, *Pandalus borealis*, is the classical pigment source in Norwegian salmon farming (Torrissen et al. 1989). This shrimp waste was usually added to wet diets (30% dry matter) at a level of 10%, giving a total dietary astaxanthin level of 10-25 mg/kg dry matter. This diet was fed the last 3-6 mo prior to slaughtering and gave, at that time, an acceptable flesh pigmentation of both Atlantic salmon and rainbow trout. It is doubtful, however, that the pigmentation regarded as acceptable 10-15 yr ago would be accepted by the market today.

Canthaxanthin has been synthesized since 1964 and soon achieved a dominant role as a pigment source for farmed salmon. This is because it was readily available and, as a dry powder, it could be added to any diet at desired levels. Usually, 50 mg of canthaxanthin was added per kg dry diet, and the farmers gradually extended the period of carotenoid feeding. This increased the pigmentation gradually and lead to a new standard flesh color in the market. When synthetic astaxanthin appeared in the market in the early 1980s, it soon took over the role of canthaxanthin as the primary carotenoid source for Atlantic salmon farming in Norway. This is because astaxanthin is the natural pigment for salmonids, and it was assumed that this pigment had a preference among the consumers. Due to a higher rate of deposition compared with canthaxanthin, the level of astaxanthin in the diet was decreased to 40 mg/kg dry diet.

In recent years, it has been difficult to satisfy the preferred flesh color in some markets; it has been claimed that Scottish-produced Atlantic salmon has a better flesh color than that from Norway. The Scottish salmon farmers are still using canthaxanthin as the pigment source for Atlantic salmon, and at a higher level (75 mg/kg) than their Norwegian colleagues. The lower level of astaxanthin in Norwegian feed, the high energy content, and the lighter color of astaxanthin compared with canthaxanthin are factors responsible for the lighter pigmentation of Norwegian as compared with Scottish salmon.

At the Institute of Marine Research, Division of Aquaculture, Matre Aquaculture Research Station, we have worked for nearly 15 yr with problems concerning pigment sources, pigment absorption and deposition and biological functions of carotenoids. In this section, we will present results on the effect of dietary carotenoid level on the deposition of astaxanthin and canthaxanthin in the flesh of salmonids. We will discuss these results in relation to the hypothetical deposition.

## DIETARY CAROTENOID LEVEL

Dietary levels of astaxanthin or canthaxanthin are a major factor determining the level of pigments in the flesh (Spinelli and Mahnken 1978; Kotik et al. 1979; Torrissen 1985). All these studies show an increased deposition of carotenoids in the flesh by increased dietary concentration of astaxanthin. Similar results are found for canthaxanthin in chicken (Tyczkowski and Hamilton 1986). However, the rate of retention usually decreases with increasing dietary level. This is illustrated in Fig. 1 where data from dose-response studies in the literature are transformed to a relative pigment deposition in flesh as a function of relative pigment concentration in the diet. From these data, it seems that a 200% increase in dietary concentration gives a 40-50% decrease in the relative pigment deposition, (Pn/Dn*100)/(Pr/Dr): where Pn = flesh carotenoid concentration at diet level n, Pr = flesh concentration in reference diet, and Dn or r = the corresponding diet concentration. This limitation may be due to reduced absorption from the diet, increased metabolism of absorbed carotenoids or limitation of the flesh deposition itself.

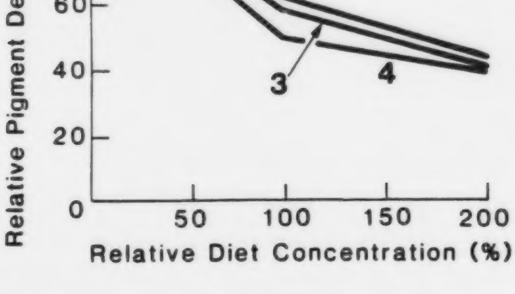


**Fig. 1. Relative pigment deposition. 1: Storebakken et al. 1987; 2: Torrissen 1985; 3: Spinelli and Mahnken 1978; 4: Kotik et al. 1979.**

In one of our studies, we measured the apparent digestibility of canthaxanthin in rainbow trout (Torrissen et al. 1989). In this study we found a linear decrease in the apparent digestibility coefficient by increasing the levels of canthaxanthin from 0-200 mg/kg dry diet (Fig. 2). Due to problems in methodology, it is difficult to propose absolute values for the apparent digestibility coefficient. The carotenoid level in the plasma is found to increase linearly with dietary concentrations in the range of 0-80 mg/kg dry diet. This indicates that factors other than digestibility are also of importance for the amount deposited in the flesh.

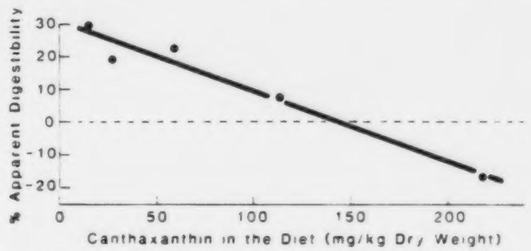


**Fig. 2. Apparent digestibility coefficients of canthaxanthin with increasing dietary levels of canthaxanthin (Torrissen et al. 1990).**

In a work by Hardy et al. (1990), it was demonstrated that there is a high excretion rate of metabolic products of canthaxanthin in the bile (Fig. 3). This is to our knowledge the first demonstration of excretion of metabolic products of carotenoids in salmonids. However, no dose-response effects were investigated in this study. Knowledge of the oxidative metabolism of carotenoids in relation to dietary level and flesh pigmentation is of fundamental importance for proposing a strategy for salmon pigmentation, and further research in this field is required.

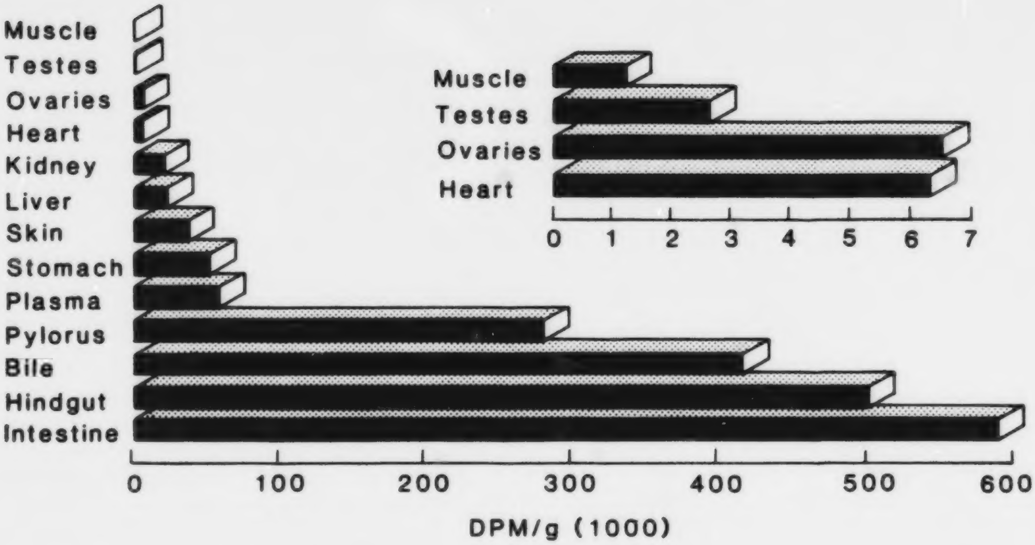


**Fig. 3. The 14C activity in sampled tissues 96 h after force feeding a semi-purified diet containing 14C-labelled canthaxanthin (DPM/G = disintegrations per minute per gram sampled) (Hardy et al. 1990).**

Our studies on interactions between deposition of astaxanthin and canthaxanthin have shown a preference for astaxanthin both in absorption and flesh deposition. So far, we have no data as to how astaxanthin is bound in the flesh, but available data suggest a maximum level of astaxanthin or canthaxanthin in the flesh (Torrissen and Torrissen 1985; Torrissen et al. 1989). The dose-response effect of dietary astaxanthin or canthaxanthin level on flesh pigmentation seems to be influenced by digestion, metabolism and the actual deposition process. There are indications that the digestibility is of major importance, but both metabolism and deposition have significant influence on the amount of carotenoids accumulated in the flesh of Atlantic salmon or rainbow trout.

## REARING MARINE LARVAE IN INTENSIVE SYSTEMS

The development program for intensive rearing of marine fish fry commenced at Austevoll Aquaculture Station in 1979, with cod as the model fish (Jensen et al. 1979). There were three main objectives within this program:

1. To develop methods for the production of large numbers of cod fry for release purposes;
2. To produce cod fry for the aquaculture industry; and
3. To develop and enhance rearing methods for marine fish fry.

The systems for intensive and extensive mass production of cod fry included components for spawning, incubation, collection and production of food organisms, net-pen experiments, intensive rearing in fibreglass tanks, and rearing in ponds.

## STARTFEEDING WITH FORMULATED DIETS IN FLOW-THROUGH TANKS

Startfeeding of marine fish larvae in culture is done mainly with live rotifers, *Artemia*, and collected plankton. This is a costly method and requires considerable expertise. Adron et al. (1974), Girin (1979), and Moksnes et al. (1989) managed to rear plaice, sole, sea bass, and wolffish beyond metamorphosis using formulated diets. The advantages of a formulated diet compared to live feed are evident, and a successful product would certainly represent a breakthrough in the cultivation of marine fishes. Since 1981, we have been developing formulated diets and evaluating these as startfeed for 4-d-old cod larvae. In 1981, eight diets were tested, four based on frozen zooplankton and four on poultry eggs. Life from hatching to starvation was increased by between 25 and 29% for larvae fed poultry egg-based diets (Huse 1981). Larvae with a substantial stomach content of poultry egg diet were transferred to a chamber without feed. After 24 h, there was hardly any change in stomach content to be observed by looking at the larvae under a microscope. As the ingredients in the feed were believed to contain all necessary nutrients for growth, digestion was suggested as the limiting factor (Huse 1981).

Based on experience from the 1981 season, two hypotheses were introduced: 1) the fish larvae are fully developed at first feeding, but need an unknown initiation substance from the feed to start digestion and growth; and 2) the fish larvae are not fully developed at first feeding, and need a growth and development promoting substance from the feed.

During the 1982 season, poultry egg diet was compared with a diet based on cod roe. The group fed a diet based on cod roe grew significantly better, but died after 16 d, as did the standard diet group based on poultry egg (Huse et al. 1982). Based on the results of the experiments in 1982, cod roe was used as a standard diet in the experimental setup in 1983 and later.

The initiation substance hypothesis was tested with prostaglandins and steroids as additives in the cod diet. Larvae fed live rotifers were used as a control. The group fed cod roe survived twice as long as the starvation group, but the weight increase was low compared with that of the larval group fed rotifers. No larvae fed the standard diet supplemented with hormones lived longer than larvae fed the standard diet without hormones. On the contrary, where there was a difference in survival, it was always in favor of the pure standard diet (Huse et al. 1983).

A very rapid leakage of water soluble substances from the small dry feed particles occurred when the feed was mixed with seawater (Garatun-Tjeldstø, pers. commun.). Based on this observation, work on coating the feed particles with fat substances to reduce leakage was conducted in 1984. During the 1985 season, it was confirmed that vitaminized cod roe, fed directly to the larvae, could be improved by addition of proteose peptone, and that stearol was a promising coating ingredient (Garatun-Tjeldstø et al. 1987). The coating prevented leakage from the feed and also improved the density and distribution of the feed.

Different roe types, such as capelin roe, herring roe, blueling roe, and cod roe were compared as the main ingredients in dry diets for cod larvae. The herring roe and cod roe gave the best growth, and also showed the best physical properties, density, and distribution in the tank. In 1987, we made wet feed of the best roe meal and compared it with dry particles. The wet feed gave longer survival and better growth. In 1988, plaice were startfed on wet roe meal, and we managed to get a few larvae through metamorphosis. However, the survival and growth was poor compared with larvae fed live diets.

Formulated diets made of cod roe give prolonged survival of cod and halibut. Growth of cod, turbot, and plaice is much slower on formulated feed than on live feed (Garantun-Tjeldstø et al. 1987). Wet feed gave better growth and longer survival than dry feed and gave metamorphosis in plaice. In cod, development of intestine and filling of the swimbladder is observed in larvae fed dry diet.

## STARTFEEDING WITH LIVE FEED IN FLOW-THROUGH SYSTEMS

### COLLECTION AND PRODUCTION OF FOOD ORGANISMS

Two types of living food organisms have been used in the pen and in the flow-through tanks. Natural plankton was collected from the sea by a double plankton net mounted on a propeller pump (Jensen et al. 1979). The collected plankton was distributed to the pens, and was the major feed source for the cod larvae until 1984. In the tanks, the collected plankton was used both as supplementary feed and as a main feed source.

Plankton concentration in the sea is variable. To ensure a stable feed supply, rotifers (*Brachionus plicatilis*) were cultured at Austevoll from 1982 according to the method described by Gatesoupe and Luquet (1982). Rotifers were used in the larval tanks and also served as supplementary feed in the pen experiments (Huse et al. 1984). Rotifers, without enrichment and enriched with different types of dry feed at different enriching time, have been tried out as startfeed for cod larvae. The influence of different water temperatures on larval growth has also been studied. The effect of adding microalgae and the effect of sampling were also tested.

## ENRICHMENT DIET

1. Fish meal, codliver oil, and egg yolk.
2. cod roe.
3. Fish meal, cod roe, codliver oil and egg yolk (75:25:5:1).

Chemical analysis showed that rotifers enriched with a mixture of fish meal and cod roe had a high content of highly unsaturated fatty acids, and an adequate content of the essential amino acids, especially methionine and cysteine, which otherwise may be insufficient in live feed (Olsen et al. 1985) (Table 1).

Table 1. Fatty acid (FA) composition and level of methionine and cysteine in *Brachionus* fed different enrichment diets. The difference is due to enrichment diet in the digestive tract of *Brachionus*.

| | *Brachionus* | | |
|---|---|---|---|
| | B11 | B22 | B33 |
| Saturated FA (%) | 23.1 | 12.2 | 19.1 |
| Monoenic FA (%) | 39.1 | 28.3 | 38.0 |
| Polyunsaturated FA (%) | 10.4 | 26.2 | 28.3 |
| Methionine (mg/g d.w.) | 9.7 | 5.2 | 8.9 |
| Cystine | 2.1 | 1.4 | 1.2 |

Rotifers fed a dry diet containing cod roe meal for more than 4 h gave the best growth of cod. Increasing the water temperature also increased the growth rate. The best result so far is two metamorphosed cod larvae per litre in open circulation tanks.

## PEN CULTURE

The pen experiments started in 1979 (Jensen et al. 1979). The idea was to develop a system that could produce cod fry with the use of collected natural plankton. The plankton collection system, as mentioned earlier, was constructed to concentrate the natural plankton occurring in the sea and, at the same time, exclude unacceptably large zooplankton. After several years of modification, the pen system functioned well. However, the survival rate of young cod beyond metamorphosis had been low (Huse et al. 1984).

It is worthy of note that the fat content of plankton falls sharply after 4 h live storage (Table 2). This is probably due to starvation of the plankton. The fat content is halved 4 h after the plankton has been collected from the sea. Zooplankton has a higher content of protein and fat, and a lower ash content when newly collected than do rotifers.

Table 2. Chemical content of plankton and enriched rotifers.

| Zooplankton | Protein | Fat | Ash |
|---|---|---|---|
| Freshly caught: | | | |
| >250 µ | 61 | 21 | 18 |
| 90-250 µ | 65 | 35 | - |
| After 4 h: | | | |
| >250 µ | 64 | 15 | 21 |
| 90-250 µ | 64 | 14 | 22 |
| Rotifers enriched with: | | | |
| fish meal | 58 | 12 | 30 |
| cod roe | 55 | 18 | 27 |
| fish + cod roe | 55 | 17 | 28 |

There are probably several factors causing the low survival rate of cod larvae fed in pen culture. These are low temperatures (about 6°C), the change in chemical composition of the zooplankton over time due to lack of food intake (phytoplankton), the distribution in the pen, and the composition of the zooplankton.

## REFERENCES

Adron, J. W., A. Blair, and C. B. Cowey. 1974. Rearing plaice (*Pleuronectes platessa*) larvae to metamorphosis using an artificial diet. Fish. Bull. 72: 353-357.

Anon. 1990. Norwegian salmon 1989 facts. Fiskeoppdretternes Salgslag A/L, Trondheim, Norway. 2 p.

Garatun-Tjeldstø, O., J. Thomassen, L. Klungsøyr, I. Opstad, B. Strand, and I. Huse. 1987. Artificial start-feed to larval rearing of sea bass (*Dicentrachus labrax*). Aquaculture 22: 149-163.

Gatesoupe, F.-J., and P. Luquet. 1982. Weaning of the sole (*Solea solea*) before metamorphosis. Aquaculture 26: 359-368.

Girin, M. 1979. Feeding problems and the technology of rearing marine fish larvae, p. 359-366. In J. E. Halver and K. Thiews (ed.) Finfish nutrition and fishfeed technology, Vol. I. H. Heenemann GmGH & Co., Berlin 42.

Hardy, R. W., O. J. Torrissen, and T. M. Scott. 1990. Absorption and distribution of 14C-labeled canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 87: 331-340.

Huse, I. 1981. An attempt to start feed cod larvae with artificial diets. Int. Counc. Explor. Sea C.M. 1981/F:14.

Huse, I., P. A. Jensen, I. Opstad, H. Otterå, and B. Strand. 1984. Intensive production of cod fry at Austevoll. Final report. Int. Counc. Explor. Sea C.M. 1984/F:33.

Huse, I., I. Opstad, and P. Jensen. 1983. Intensive production of cod fry systems and results so far. Int. Counc. Explor. Sea C.M. 1983/F:24.

Huse, I., I. Opstad, L. Klungsøyr, and B. Walter. 1982. Endocrine and nutritional factors affecting the first food uptake by cod larvae. Int. Counc. Explor. Sea C.M. 1982/F:17.

Jensen, P. A., B. Braaten, and D. Møller. 1979. Rearing of cod fry in plastic pens. Int. Counc. Explor. Sea C.M. 1979/F:29.

Kotik, L. V., G. Yu. Tolokonnikov, and V. N. Dubrovin. 1979. The effect of krill meal additions to feeds on muscle pigmentation in rainbow trout (*Salmo gairdneri*). J. Ichthyol. 19: 119-123.

Moksness, E., J. Gjøsæter, A. Reinert, and I. S. Fjallstein. 1989. Start-feeding and on-growing of wolffish (*Anarhichas lupus*) in the laboratory. Aquaculture 77: 221-228.

Olsen, Y., J. A. Heldal, G. Lambertsen, H. Reinertsen, G. Rosenlund, K. Sandnes, B. Strand, O. Sæther, and K. M. Vårum. 1985. Kjemisk sammensetning av levendefòr. SINTEF Rapp. STF21 A 85074, 27 p.

Spinelli, J., and C. Mahnken. 1978. Carotenoid deposition in pen-reared salmonids fed diets containing oil extracts of red crab (*Pleuroncodes planipes*). Aquaculture 13: 213-233.

Storebakken, T., P. Foss, K. Schiedt, E. Austreng, S. Liaaen-Jensen, and U. Manz. 1987. Carotenoids in diets for salmonids - IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. Aquaculture 65: 279-292.

Torrissen, K. R., and O. J. Torrissen. 1985. Protease activities and carotenoid levels during the sexual maturation of Atlantic salmon (*Salmo salar*). Aquaculture 50: 113-122.

Torrissen, O. J. 1985. Pigmentation of salmonids: factors affecting carotenoid deposition in rainbow trout. Aquaculture 46: 133-142.

Torrissen, O. J., R. W. Hardy, K. D. Shearer, and T. M. Scott. 1990. Effect of dietary canthaxanthin level and lipid level on apparent digestibility coefficients for canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). Aquaculture (in press).

Torrissen, O. J., R. W. Hardy, and K. D. Shearer. 1989. Pigmentation of salmonids - carotenoid deposition and metabolism. CRC Critical Reviews in Aquatic Sciences 1: 209-225.

Tyczkowski, J. K., and P. B. Hamilton. 1986. Canthaxanthin as a model for the study of utilization of oxycarotenoids by chickens. Poult. Sci. 65: 1350-1356.

# CONTROL OF THE TIMING OF SMOLTIFICATION IN ATLANTIC SALMON: ENDOGENOUS RHYTHMS AND ENVIRONMENTAL FACTORS

J. Duston and R. L. Saunders
Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick E0G 2X0 Canada

## ABSTRACT

Duston, J., and R. L. Saunders. 1990. Control of the timing of smoltification in Atlantic salmon: endogenous rhythms and environmental factors, p. 99-105. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The completion of smolting, and several weeks later the subsequent loss of smolt characteristics, is controlled by an endogenous circannual rhythm entrainable by changes in photoperiod. In potential smolts, abrupt increases in photoperiod from October onwards advance the completion of smoltification. Gill $Na^+K^+$ATPase activity as measured *in vitro* is stimulated by increases in photoperiod and, although usually associated with salinity tolerance, is dissociable from the latter. The photoperiod cued completion of smolting is stimulated by increases in temperature, but not by constant high temperature. Declines in ATPase activity and salinity tolerance in smolts retained in fresh water are accelerated by temperatures ≥10°C.

## RÉSUMÉ

Duston, J., and R. L. Saunders. 1990. Control of the timing of smoltification in Atlantic salmon: endogenous rhythms and environmental factors, p. 99-105. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'accession à l'état de smolt et, plusieurs semaines plus tard, la perte des caractéristiques de cet état sont régies par un rythme circannuel endogène induit pas des changements dans la photopériode. Des augmentations très marquées dans la photopériode à partir du mois d'octobre accélèrent le processus de smoltification chez les futurs saumoneaux. L'activité de la $Na^+K^+$ATPase des branchies mesurée *in vitro* est stimulée par des augmentations dans la photopériode et, bien que cette activité soit généralement associée à la tolérance à la salinité, elle peut être dissociée de celle-ci. L'accession à l'état de saumoneau induit par la photopériode est stimulée par les augmentations de température et non par des températures constamment élevées. Les baisses enregistrées dans l'activité de l'ATPase et dans la tolérance à la salinité chez les saumoneaux retenus en eau douce sont accélérées par les températures supérieures ou égales à 10°C.

## INTRODUCTION

Smolting in Atlantic salmon (*Salmo salar* L.) involves a series of physiological and behavioral changes which appear to commence in the summer, with potential smolts exhibiting an increase in growth rate, and concludes the following spring with the development of salinity tolerance and migration to sea. Under natural conditions, completion of smoltification is correlated with increasing photoperiod and temperature. The present paper summarizes the results of experiments which investigate the role of photoperiod, temperature and endogenous rhythmicity in the completion of smoltification in Atlantic salmon.

## EXPERIMENT 1

An abrupt increase in photoperiod following the winter solstice serves as an entrainment cue which phase advances a circannual rhythm of smoltification.

## BACKGROUND

This experiment has been reported elsewhere (Duston and Saunders, 1990). The annual cycle of daylength has an important influence on the timing of smoltification in a number of salmonid species (Saunders and Henderson 1970; Wagner 1974; Komourdjian et al. 1976; Clarke et al. 1978, 1985; Brauer 1982; Lundqvist 1983; McCormick et al. 1987). Hoar (1965) proposed that the role of photoperiod in smoltification is to synchronize an endogenously driven seasonal cycle in osmotic and ionic regulation. This hypothesis was supported by Eriksson and Lundqvist (1982) who observed that Baltic salmon maintained under constant environmental conditions exhibited a circannual cycle of smoltification. Endogenously driven circannual rhythms have been observed in some species of fish and other vertebrates (Gwinner 1986). Rainbow trout held under unchanging photoperiod and temperature exhibit a free-running circannual rhythm of gonadal maturation which is entrainable by changes in the daylength (Duston and Bromage 1988). The present study tests the hypothesis that an abrupt increase in daylength following the winter solstice serves as an entrainment cue which determines the magnitude of an advance in the timing of the completion of smoltification.

## METHODS

Six groups of n=200 potential 1+ smolts were each allocated to light-proof 1 m$^2$ tanks. All groups (except group LDN) were maintained on a winter solstice photoperiod until at ca. 30-d intervals the daylength in each group was increased to LD 16:8 (Fig. 1). Group E (control) was maintained on winter solstice photoperiod throughout. Temperature was held at 10.0°C (±0.2°C) until June, when the ambient supply rose above 10°C. At 2- to 4-wk intervals, a random sample of 10 fish from each group was subjected to 96 h, 37.5 o/oo salinity tolerance test; the number of fish alive at 96 h was expressed as percent survival. Brownlee's test (Brownlee 1960) was used to compare percent survival in the salinity tolerance tests.

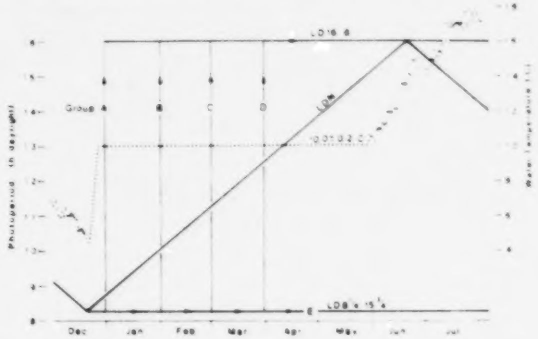


**Fig. 1. Experiment 1 photoperiod regimes and temperature. Groups A-E were maintained under LD 8 1/4:15 3/4 from Dec. 21 onwards until in groups A-D it was increased and held on LD 16:8 from the dates shown.**

## RESULTS AND CONCLUSIONS

The salinity tolerance tests indicate that smoltification was completed in sequence according to when each group received the increase in photoperiod (Fig. 2). Groups A and B exhibited high salinity tolerance in late February, significantly advanced compared with groups C, D and LDN, which developed high salinity tolerance in mid-March, mid-April and May, respectively. Importantly, Group E also developed high salinity tolerance without experiencing any increase in photoperiod or temperature, supporting the hypothesis that the

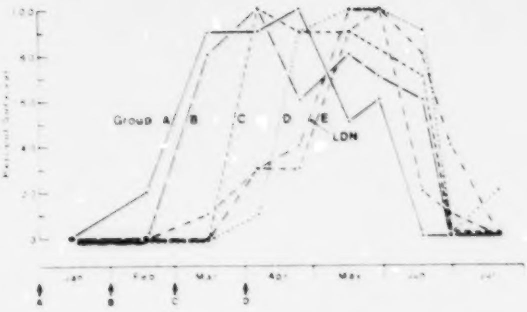


Fig. 2. Effect of Experiment 1 photoperiod regimes on percent survival of fish (n=10) subjected to 96-h, 37.5 o/oo salinity tolerance tests.

completion of smoltification, as judged by salinity tolerance, is controlled by a circannual rhythm or 'clock'. The abrupt increase in photoperiod experienced by groups A-D served as an entrainment cue or zeitgeber which advanced the timing of completion of smoltification. It is proposed that the 'premature' arrival of a long daylength was perceived by a circannual clock within the fish as 'running behind time', which responded with a compensatory phase advance in the completion of smolting. In each group, high salinity tolerance was maintained for only a period of weeks and then declined. In groups A, B and C, the loss of salinity tolerance occurred under unchanging photoperiod and temperature, indicating that the period of high salinity tolerance was under endogenous circannual control. In other groups, the loss of salinity tolerance occurred rapidly in June in association with the rise in water temperature (see Experiment 3).

## EXPERIMENT 2

Increase of photoperiod or temperature in winter advances completion of some aspects of smoltification.

## BACKGROUND

This experiment has been reported elsewhere (Duston et al. 1989). Experiment 1 showed that an increase in photoperiod can advance the timing of smoltification at constant 10°C. Temperature is believed to play an important role in the completion of smoltification by controlling the rate of the physiological response to photoperiod (Hoar 1988). Experiment 2a investigates if an increase in photoperiod in October can result in an advancement of smoltification under low ambient temperatures. Experiment 2b provides a more direct test of the hypothesis that temperature controls the rate of physiological response to photoperiod by quantifying the development of smolting in a group of fish subjected to an increase in temperature to 10°C in February compared with ambient temperature controls.

## METHODS

### Experiment 2a: Effect of increase in photoperiod

On Oct. 11, potential smolts (fork length ≥11.0 cm) were divided into two groups (n=300), each maintained in a 2-m diameter light-proof tank supplied with fresh water at ambient temperature. One group was maintained under LD 16:8. The control group was maintained under LDN. Duration of feeding and amount of feed (% body weight $day^{-1}$) were the same in both groups.

### Experiment 2b: Effect of increase in temperature

On Feb. 6, potential smolts (fork length ≥12.0 cm) were divided into two groups (n=110), each maintained in a 1-$m^2$ light-proof tank supplied with fresh water under an LDN photoperiod. In group 1, the temperature was raised by 2°C $day^{-1}$ until it reached 10°C at which it was maintained. In the control group, temperature was ambient.

### Gill ATPase activity

Fish (n=8-10) were sampled at regular intervals from each group; gill filaments were removed and frozen in buffer at -80°C until analyzed for ATPase activity using the method of McCormick et al. (1987) except incubation temperature was 20°C.

### Salinity tolerance

96-h, 37.5 o/oo tolerance tests were performed at the experimental freshwater temperature. Brownlee's test and ANOVA were used to analyze results.

## RESULTS AND CONCLUSIONS

### Experiment 2a

LD 16:8 from Oct. 11 onwards advanced the timing of smolting. By December the LD 16:8 group exhibited significantly elevated salinity tolerance and ATPase activity compared with controls (Fig. 3). Similar to Experiment 1, high salinity tolerance was only maintained for a period of weeks before declining. In contrast to Experiment 1, the development of salinity tolerance between November and January was inconsistent and attained a maximum of only 60% survival, possibly due to the low temperatures, or perhaps because in October only a certain percentage of the fish were physiologically competent to respond to the increase in photoperiod. Importantly, in the LD 16:8 group, the decline in salinity tolerance in January-February was not accompanied by a decline in ATPase activity which remained elevated for the duration of the experiment, indicating that: a) a long or increasing photoperiod stimulates gill ATPase activity; and b) elevated gill ATPase activity as measured *in vitro* forms (at most) only part of the hypo-osmoregulatory system enabling fish to survive high salinities, in agreement with a recent study on rainbow trout (Madsen and Naamansen 1989).

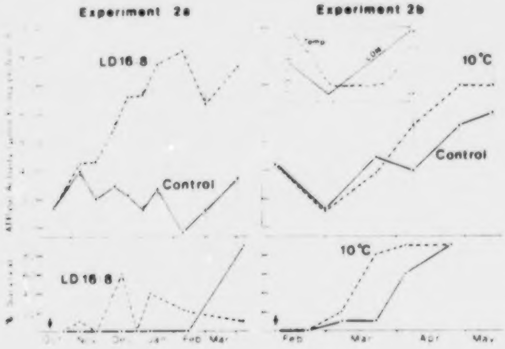


**Fig. 3. Effect of an increase in photoperiod to LD 16:8 (Experiment 2a), or an increase in temperature to 10°C (Experiment 2b) on gill ATPase activity ($\bar{X}$ ± S.E.; n=8) and percent survival to 96-h, 37.5 o/oo salinity tolerance tests. ↓ indicates start of treatment. Inset: Control photoperiod and temperature.**

### Experiment 2b

Increases in ATPase activity occurred in both 10°C and control groups from late February onwards (Fig. 3). No significant differences in ATPase activity were detected between groups but the 10°C group had higher mean levels from April onwards. The increase in temperature resulted in a significant advance in the timing of salt water tolerance, but the controls also showed a small increase in salinity tolerance during March at the same time as the 10°C group. These results support the hypothesis that the increase in temperature in the spring accelerates the physiological response cued by the increase in photoperiod. However, the stimulatory effect of an elevation in temperature appears to be complex, and not simply due to it increasing metabolic rate, e.g. in Experiment 1, maintaining pre-smolts under LDN and 10°C from December onwards resulted in high salinity tolerance being exhibited in May (Fig. 2), significantly delayed compared to the present experiment where the LDN controls under lower ambient temperature developed high salinity tolerance by mid-April. There is some evidence that fish raised on farms with a well water supply (8-10°C) exhibit a delayed development of smolt status in the spring (pers. obs.). Overall, these observations and others (Wagner 1974; Jonsson and Ruud-Hansen 1985) suggest that completion of smolting is stimulated by an increase in temperature rather than by absolute temperature *per se*.

## EXPERIMENT 3

Effect of increases in water temperature on gill $Na^+K^+$ATPase activity and salinity tolerance in smolts.

### BACKGROUND

Experiments 1 and 2 indicate that increases in temperature in spring stimulate the completion of smolting which is entrained by the annual photoperiod cycle. However, in smolts that are retained in fresh water (FW), further rises in temperature have been correlated with "desmoltification" (Hoar 1988). Studies on *Oncorhynchus* species have associated increases in temperature with reductions in gill $Na^+K^+$ATPase (ATPase) activity (Zaugg and McLain 1972, 1976; Adams et al. 1973; Zaugg and Wagner 1973). Experiment 3 determines the effect of increases in temperature on ATPase activity and salinity tolerance in Atlantic salmon smolts.

### METHODS

Potential 2+ smolts were maintained in FW under natural temperature and LDN photoperiod until they completed smolting in spring 1989. On May 2, the smolts were randomly divided into four groups (n=80 per group/tank). In three groups, the FW temperature was raised over a 3- to 4-h period to either 10, 13 or 16°C, the fourth group was at ambient temperature (Fig. 4). At regular intervals, gill filaments were removed from a random sample (n=8) in each group for analysis of ATPase activity. Also, random samples of fish from each group were subjected to 96-h, 37.5 o/oo salinity tolerance tests performed at the respective FW experimental temperature.

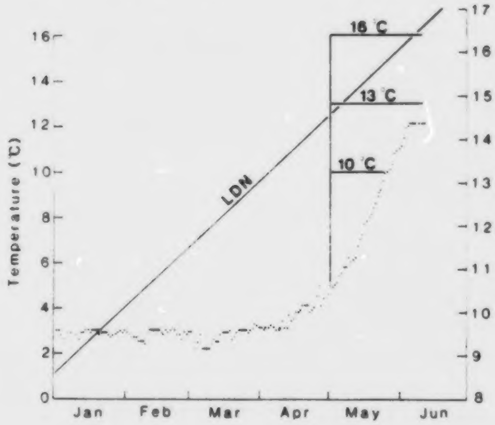


**Fig. 4. Experiment 3 temperature regimes and photoperiod. Temperature increased on May 2.**

## RESULTS AND CONCLUSIONS

The greater the increase in temperature, the greater was the decline in ATPase activity, with the 16°C group falling to pre-smolt ATPase activity levels approximately 20 d after the start of the experiment (Fig. 5). The results indicate that for these fish, temperatures ≥10°C cause a decline in ATPase activity. However, Atlantic salmon smolts can be produced at rearing temperatures up to 16°C (Johnston and Saunders 1981), and fish raised at constant 14°C completed smolting and exhibited characteristic elevations in ATPase activity (Duston and Saunders, unpubl. data). Similar to the conclusions drawn from studies on upper lethal temperatures in fish (Fry 1947), it appears that the effect of a particular temperature depends upon the fishes previous thermal history. For example, the data of Adams et al. (1973) on steelhead trout show that 10°C can either inhibit or stimulate gill ATPase, depending whether the previous rearing temperature was higher or lower than 10°C.

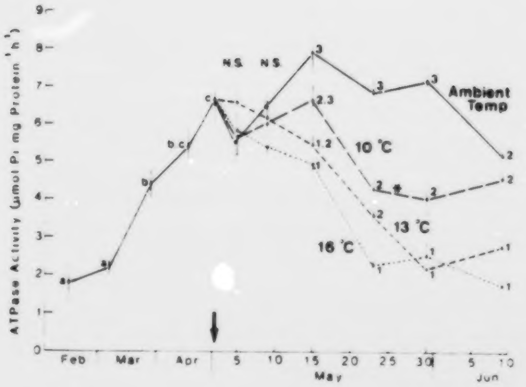


**Fig. 5. Experiment 3 gill ATPase activity ($\bar{x}$ ± S.E.; n=8). ↓ indicates start of treatment. Letters indicate $P \leq 0.05$ differences with respect to time; numbers indicate intergroup $P \leq 0.05$ differences. * = time when 10°C group became ambient temperature.**

The mechanism of temperature inhibition of gill ATPase is not direct since *in vitro* studies have indicated that the optimal temperature for this enzyme in salmonids is ca. 40°C (Giles and Vanstone 1976). As proposed by Foskett et al. (1983), it is suggested that the observed alterations in ATPase activity are determined by the effects of hormones (possibly prolactin or cortisol) on chloride cell differentiation, with the observed 9- to 13-d time lag between the increase in temperature and reduction in ATPase corresponding well with the estimated turnover rate of chloride cells (Conte and Lin 1967).

The increase in salinity tolerance in the spring (Fig. 6) was associated with increases in

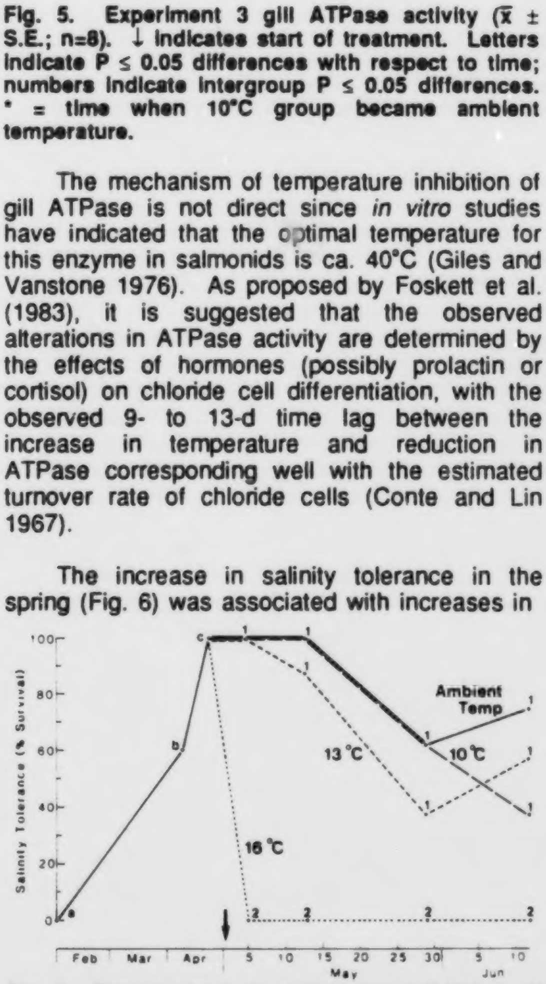


**Fig. 6. Experiment 3 percent survival if fish (n=8) subjected to 96-h, 37.5 o/oo salinity tolerance tests. ↓ indicates start of treatment. Letters indicate $P \leq 0.05$ differences with respect to time; numbers indicate intergroup $P \leq 0.05$ differences.**

ATPase activity, confirming that under normal hatchery conditions both these parameters are good indicators of the completion of smolting (Boeuf et al. 1985; McCormick et al. 1987). However, after the start of the experiment, and similar to Experiment 2, there was a breakdown in the correlation between these two factors. The significant differences in ATPase levels between control, 10 and 13°C groups were not accompanied by similar differences in salinity tolerance, e.g. in late May/June, the 13°C group had basal ATPase activity and yet exhibited 40-60% survival in the tolerance tests. High salinity tolerance in the absence of elevated gill ATPase activity has been observed previously (Saunders and Harmon, in press). The 16°C group showed a rapid decline in salinity tolerance; however, the results are confounded by the fact that the tests were performed at the experimental FW temperature (16°C); on May 13, two salinity tolerance tests from the 16°C group run at 13 and 16°C resulted in 100 and 0% survival, respectively. Overall, the results emphasize the need for a more detailed study of the relationship between gill ATPase activity, salinity tolerance and temperature in Atlantic salmon.

## REFERENCES

Adams, B. L., W. S. Zaugg, and L. R. McLain. 1973. Temperature effect on parr-smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium-potassium stimulated triphosphatase. Comp. Biochem. Physiol. 44: 1333-1339.

Boeuf, G., A. LeRoux, J. L. Gaignon, and Y. Harache. 1985. Gill (Na+-K+)-ATPase activity and smolting in Atlantic salmon (*Salmo salar* L) in France. Aquaculture 45: 73-81.

Brauer, E. P. 1982. The photoperiod control of coho salmon smoltification. Aquaculture 28: 105-111.

Brownlee, K. A. 1960. Statistical theory and methodology of science and engineering. John Wiley and Sons, Inc., New York, 570 p.

Clarke, W. C., H. Lundqvist, and L. O. Eriksson. 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon *Salmo salar* L. J. Fish Biol. 26: 29-35.

Clarke, W. C., J. E. Shelbourn, and J. R. Brett. 1978. Growth and adaptation to seawater in 'underyearling' sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon subjected to regimes of constant or changing temperature and day length. Can. J. Zool. 56: 2413-2421.

Conte, F. P., and D. H. Y. Lin. 1967. Kinetics of cellular morphogenesis in gill epithelium during seawater adaptation of euryhaline *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 23: 945-957.

Duston, J., and N. Bromage. 1988. The entrainment and gating of the endogenous circannual rhythm of reproduction in the female rainbow trout (*Salmo gairdneri*). J. Comp. Physiol. 164: 259-268.

Duston, J., and R. L. Saunders. 1990. The entrainment role of photoperiod on hypo-osmoregulatory and growth related aspects of smolting in Atlantic salmon (*S. salar*). Can. J. Zool. 68: 707-715.

Duston, J., R. Saunders, P. Harmon, and D. Knox. 1989. Increases in photoperiod and temperature in winter advance aspects of smoltification in Atlantic salmon. Bull. Aquaclt. Assoc. Can. 3: 19-21.

Eriksson, L. O., and H. Lundqvist. 1982. Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). Aquaculture 28: 113-121.

Foskett, J. K., H. A. Bern, T. E. Machen, and M. Conner. 1983. Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol. 106: 255-281.

Fry, F. E. J. 1947. Effects of environment on animal activity. Publ. Ontario Fish. Res. Lab. 55: 1-62.

Giles, M. A., and W. E. Vanstone. 1976. Changes in ouabain sensitive adenosine triphosphatase activity in gills of coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation. J. Fish. Res. Board Can. 33: 54-62.

Gwinner, E. 1986. Circannual rhythms. Zoophysiology Vol. 18. Springer, Berlin. 154 p.

Hoar, W. S. 1965. The endocrine system as a chemical link between the organism and its environment. Trans. R. Soc. Can. Ser. IV 3: 175-200.

Hoar, W. S. 1988. The physiology of smolting in salmonids, p. 275-343. In W. S. Hoar and D. J. Randall (ed.) Fish Physiology, Vol. 11B. Academic Press.

Johnston, C. E., and R. L. Saunders. 1981. Parr-smolt transformation of yearling Atlantic salmon (*Salmo salar*) at several rearing temperatures. Can. J. Fish. Aquat. Sci. 38: 1189-1198.

Jonsson, B., and J. Ruud-Hansen. 1985. Water temperature as a primary influence on timing of seaward migration of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 42: 593-595.

Komourdjian, M. P., R. L. Saunders, and J. C. Fenwick. 1976. Evidence for the role of growth hormone as a part of a 'light-pituitary axis' in growth and smoltification of Atlantic salmon (*Salmo salar*). Can. J. Zool. 54: 544-551.

Lundqvist, H. 1983. Precocious sexual maturation and smolting in Baltic salmon (*Salmo salar* L.); photoperiodic synchronization and adaptive significance of annual biological cycles. Ph.D. Thesis. University of Umea, Sweden. 75 p.

Madsen, S. S., and E. T. Naamansen. 1989. Plasma ionic regulation and gill $Na^+K^+$ATPase changes during rapid transfer to sea water of yearling rainbow trout, *Salmo gairdneri*: time course and seasonal variation. J. Fish Biol. 34: 829-840.

McCormick, S. D., R. L. Saunders, E. B. Henderson, and P. R. Harmon. 1987. Photoperiod control of parr-smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill $Na^+,K^+$ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquat. Sci. 44: 1462-1468.

Saunders, R. L., and P. R. Harmon. 1990. Influence of photoperiod on growth of juvenile Atlantic salmon and development of salinity tolerance during winter-spring. Trans. Am. Fish. Soc. (in press).

Saunders, R. L., and E. B. Henderson. 1970. Influence of photperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 27: 1295-1311.

Wagner, H. H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219-234.

Zaugg, W. S., and L. R. McLain. 1972. Changes in gill adenosine triphosphatase activity associated with parr-smolt transformation in steelhead trout, coho and spring chinook salmon. J. Fish. Res. Board Can. 29: 167-171.

Zaugg, W. S., and L. R. McLain. 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*O. kisutch*). Comp. Biochem. Physiol. 54A: 419-421.

Zaugg, W. S., and H. H. Wagner. 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comp. Biochem. Physiol. 45B: 955-965.

# PRODUCTION OF JUVENILE ATLANTIC SALMON (*SALMO SALAR*) AT THE MACTAQUAC ACCELERATED REARING FACILITY, NEW BRUNSWICK, CANADA

G. J. Farmer, P. D. Hubley, H. Jansen,
J. W. McAskill[1] and G. B. Robbins[2]
Freshwater and Anadromous Division
Department of Fisheries and Oceans
P. O. Box 550
Halifax, Nova Scotia B3J 2S7 Canada

[1] Mactaquac Fish Culture Station, R. R. #6, Fredericton, N. B. E3B 4X7.
[2] Authors listed in alphabetical order.

## ABSTRACT

Farmer, G. J., P. D. Hubley, H. Jansen, J. W. McAskill, and G. B. Robbins. 1990. Production of juvenile Atlantic salmon (*Salmo salar*) at the Mactaquac accelerated rearing facility, New Brunswick, Canada, p. 107-118. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The Department of Fisheries and Oceans, in cooperation with the New Brunswick Electric Power Commission, constructed a facility for the accelerated rearing of Atlantic salmon parr (*Salmo salar*) at the Mactaquac Generating Station on the Saint John River. River water used to cool the thrust bearings and stators of the six generating units is collected in two pipelines and flows by gravity to a pump building located adjacent to the station. A supply of cooler (not warmed) river water collected from two of the penstocks also flows by gravity to the pump building in a single pipeline. All of the warm water enters a mixing chamber in the pump building while entry of the cooler water is regulated by an automatic, butterfly valve operated by a pneumatic, temperature controller. The mixed, warm water then flows to a sump chamber in the building where it is pumped 200 m to a headtank building by a number of vertical turbine pumps. The warm water which enters the headtank building passes through an automated sand filter and then through 30 packed columns before discharging into a concrete head tank. The filter reduces the total suspended solids concentration of the warm water and the packed columns reduce total dissolved gas pressure. The filtered warm water then flows by gravity to the incubation building or to four aquadomes. Water which enters the incubation building passes through two ultraviolet water sterilizers and then to a number of upwelling incubation boxes which contain eyed eggs and alevins. Swim-up fry are removed from the upwelling boxes during early April and transferred to 3-m fiberglass tanks enclosed in the aquadomes. The aquadomes are prefabricated greenhouses installed on concrete foundations. Each aquadome contains 18 of the 3-m tanks. Use of the warm water enables production of 720,000 2-g parr by mid-June of each year, 67-71 d after initiation of feeding.

## RÉSUMÉ

Farmer, G. J., P. D. Hubley, H. Jansen, J. W. McAskill, and G. B. Robbins. 1990. Production of juvenile Atlantic salmon (*Salmo salar*) at the Mactaquac accelerated rearing facility, New Brunswick, Canada, p. 107-118. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Le ministère des Pêches et des Océans, en collaboration avec la Commission d'énergie électrique du Nouveau-Brunswick, a construit une installation pour l'élevage accéléré des tacons de saumon de l'Atlantique (*Salmo salar*) à la station génératrice de Mactaquac sur la rivière Saint-Jean. L'eau de la rivière qui est utilisée pour refroidir les paliers de poussée et les parties fixes des six génératrices est recueillie dans deux canalisations et elle est acheminée grâce à la force de gravité jusqu'à un poste de pompage adjacent à la station. L'eau de la rivière - plus froide (non réchauffée) - recueillie par deux conduites forcées est aussi acheminée par la force de gravité jusqu'au poste de pompage par une canalisation unique. Toute l'eau chaude pénètre dans un caisson de mélange situé dans le poste de pompage, tandis que l'afflux d'eau froide est réglé par une vanne-papillon automatique qui est actionnée par un régulateur pneumatique de température. Les eaux chaudes mélangées sont alors dirigées jusqu'à un bassin de déversement dans la station où l'eau est pompée sur une distance de 200 m jusqu'à un collecteur de tête par un certain nombre de pompes à turbines verticales. Les eaux chaudes qui pénètrent dans le collecteur de tête passent au travers d'un filtre au sable automatisé et ensuite au travers de 30 colonnes à corps de remplissage avant de se déverser dans un réservoir de béton. Le filtre réduit la concentration totale de solides en suspension dans l'eau chaude, et les colonnes à corps de remplissage réduisent la pression totale des gaz dissous. Les eaux chaudes filtrées sont dirigées grâce à la force de gravité jusqu'au bâtiment d'incubation ou jusqu'à quatre aquadômes. Les eaux qui pénètrent dans le bâtiment d'incubation passent au travers de deux stérilisateurs aux ultraviolets et ensuite par un certain nombre de cages d'incubation en eaux de remontée qui contiennent des oeufs embryonnés et des alevins. Les alevins qui sont capables de remonter à la surface de l'eau et d'utiliser leur vessie natatoire sont retirés des cages d'incubation en eaux de remontée au début d'avril et transférés dans des viviers en fibre de verre de trois mètres de diamètre qui sont contenus dans les aquadômes. Les aquadômes sont en fait des serres préfabriquées reposant sur des fondations de béton. Chaque aquadôme contient 18 de ces viviers de 3 mètres de diamètre. L'utilisation de l'eau chaude permet la production de 720 000 tacons de deux grammes avant la mi-juin de chaque année, c'est-à-dire de 67 à 71 jours après le début de l'alimentation.

## INTRODUCTION

The Saint John River is one of the larger North American rivers draining to the Atlantic Ocean. Its drainage area of 54,930 km$^2$ consists of a portion of northern Maine in the United States and adjacent areas of the provinces of Quebec and New Brunswick in Canada. The river has a length of 676 km and there is a 481-m decline in elevation from its source to the head of tide (Ruggles and Watt 1975). During the early history of Canada, the river provided a transportation route to the interior of New Brunswick. As the population of the Saint John River valley increased, water quality began to deteriorate as a result of land clearing and later from the development of hydroelectric dams and from industrial, agricultural, and domestic pollution. Most of the available head of the Saint John River is now utilized for hydroelectric purposes (Ruggles and Watt 1975).

The largest and most recently constructed hydroelectric dam on the Saint John River was completed in 1968 at Mactaquac which is located about 3 km above the head of tide. The project not only created a reservoir 97 km in length, resulting in the loss of a significant area of salmon habitat, but also had the potential to eliminate populations of fish that normally migrated to areas of the river above the Mactaquac Dam. Thus, fish collection facilities were incorporated in the dam and a fish culture station for rearing juvenile Atlantic salmon was constructed 2.5 km downstream to compensate for losses in salmon production.

The Mactaquac Fish Culture Station (FCS) had the capacity to produce 235,000 2-yr-old Atlantic salmon smolts when it was completed in 1968. During 1983/84, an Accelerated Rearing Facility was constructed to utilize the warm waste water from the generating units at the Mactaquac Generating Station. The development of eyed eggs and alevins is accelerated by the use of the warm water so that feeding begins during April, 2 mo earlier than had been possible in the past. Parr are reared in the warm waste water for 2 mo and then transferred, during June, to the Mactaquac FCS where they are reared for an additional 11 mo. Production at the station is now 375,000 1-yr-old salmon smolts. In this report we describe the Accelerated Rearing Facility and provide details of the fish culture methods employed at that location.

## MATERIALS AND METHODS

The Department of Fisheries and Oceans, in cooperation with the New Brunswick Electric Power Commission, designed and constructed a facility located at the Mactaquac Generating Station on the Saint John River for the accelerated rearing of Atlantic salmon parr. Civil and mechanical aspects of the facility design were completed by the Engineering Services Section, Department of Fisheries and Oceans, Halifax[3] while the electrical design was completed by New Brunswick Electric Power Commission engineers[4]. Detailed drawings of the facility are in the possession of the Engineering Services Section, Halifax. Biological input during the design process regarding water quality objectives and treatment, salmon rearing plans, and hatchery operations were provided by Department of Fisheries and Oceans biologists and technicians[5].

## RESULTS AND DISCUSSION

### MACTAQUAC ACCELERATED REARING FACILITY

The warm waste water from six 100-megawatt generating units is collected in two 30-cm diameter, polyvinyl chloride (PVC) pipelines and flows by gravity to the mixing chamber of the pump building situated adjacent to the generating station (Fig. 1). Each pipeline is connected to the stator and thrust bearing cooling systems of three generating units. A supply of cooler (not warmed) river water is collected from two penstocks and delivered by gravity to the pump building in one 30-cm diameter steel pipeline. The generating station is operated so that at least one generating unit is operating on minimum load and another unit on synchronous condense. Each generating unit provides a temperature increase of up 10°C to the river water used for cooling. Temperature increase imparted to the cooling water provided to a particular generating unit is dependent upon flow rate of the cooling water and upon the load on that unit.

---

[3] P. O. Box 550, Halifax, N. S. B3J 2S7.

[4] 515 King St., Fredericton, N. B. E3B 4X1.

[5] P. O. Box 550, Halifax, N. S. B3J 2S7 and Mactaquac Fish Culture Station, R. R. #6, Fredericton, N. B. E3B 4X1.

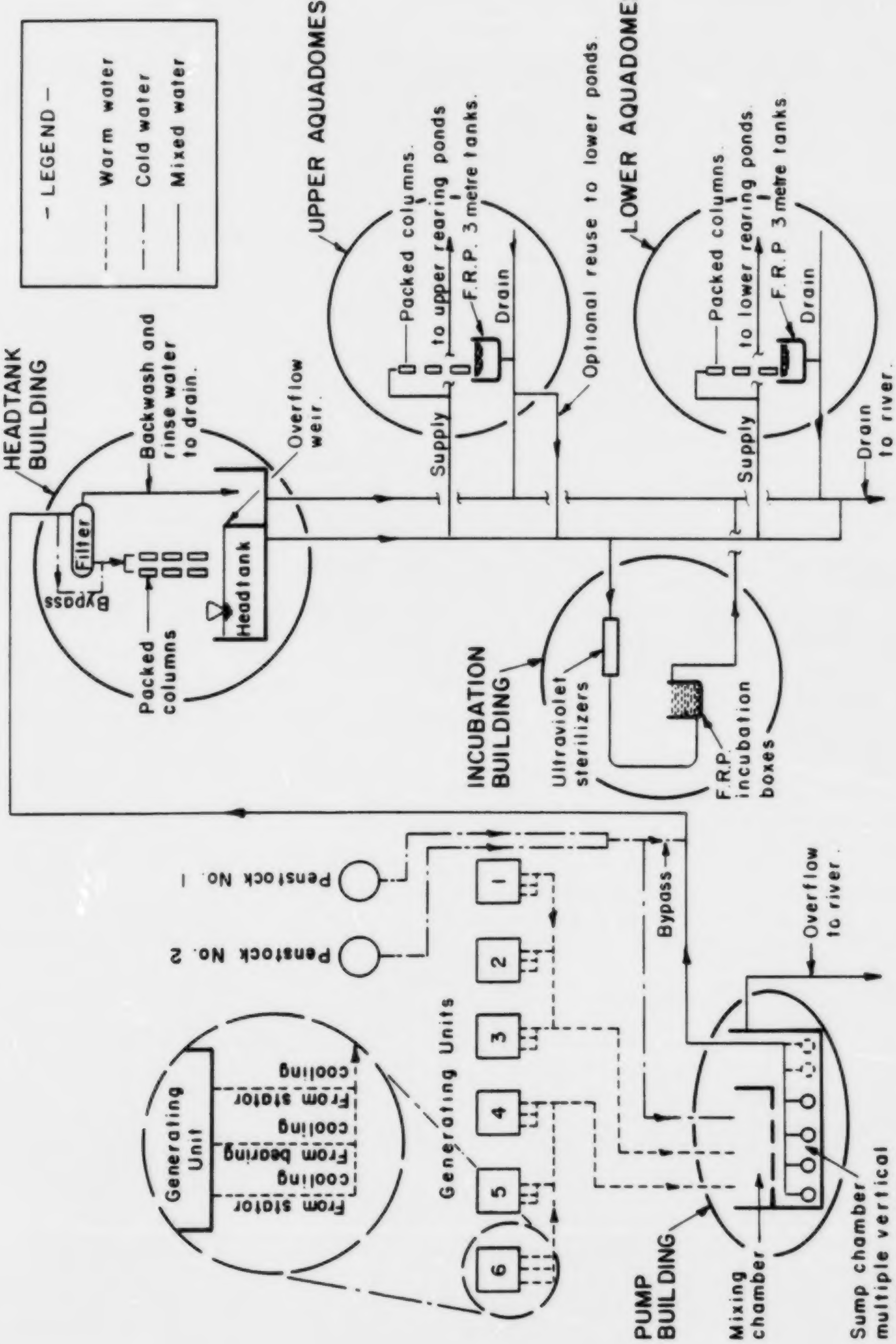

– LEGEND –
Warm water
Cold water
Mixed water
HEADTANK BUILDING
Backwash and rinse water to drain.
Overflow weir.
Filter
Bypass
Packed columns
Headtank
UPPER AQUADOMES
Supply
Packed columns
to upper rearing ponds
F.R.P. 3 metre tanks.
Drain
Optional reuse to lower ponds.
LOWER AQUADOMES
Supply
Packed columns
to lower rearing ponds.
F.R.P. 3 metre tanks
Drain
Drain to river.
INCUBATION BUILDING
Ultraviolet sterilizers
F.R.P. incubation boxes
Penstock No. 1
Penstock No. 2
Bypass
Overflow to river.
Generating Units
1
2
3
4
5
6
Generating Unit
From stator
cooling
From bearing
cooling
From stator
cooling
PUMP BUILDING
Mixing chamber
Sump chamber multiple vertical turbine pumps.

Fig. 1. Flow diagram of accelerated rearing facility.

Warm water enters the mixing chamber through one or both warm-water lines while entry of the cooler water is controlled by a 20-cm, automatic control or butterfly valve operated by a pneumatic temperature controller. The control valve opens or closes to minimize the difference between the desired and actual temperature of the mixed water. The mixed, warm water flows to the sump chamber of the pump building where provisions for the installation of six, vertical turbine pumps have been made. The pumps have been installed to prevent vortexing and unnecessary increases in the total dissolved gas pressure of the mixed water. Because water requirements for rearing vary during the January-June period, multiple pumps with different capacities were selected to meet the requirements. This minimizes pumping costs and ensures the continuous flow of water when a pump malfunctions. Mixed, warm water is pumped 200 m to the headtank building through a 45-cm diameter, high-density, polyethylene pipe. This material accommodates expansion and contraction changes and horizontal and vertical curves without the use of fittings and its low thermal conductivity limits loss of heat from the warm water. The water level in the head tank is 10 m higher than in the pump building. The pumps are automatically turned off during a number of emergency conditions and cooler, river water is delivered by gravity flow to the headtank building. The warm waste water cannot be supplied to the head tank by gravity flow.

The headtank building consists of a room which contains a sand filter and one that contains a head tank and equipment for reducing the total dissolved gas pressure of the water. The filter room is insulated and heated to conserve heat loss and protect the filter's electric and pneumatic controls from freezing while the headtank room is unheated. The warm water passes through an automated, horizontal pressure filter and then through a system of pipe headers to 30 packed columns before it discharges into the concrete head tank. The filter, which has a diameter of 2.4 m and length of 7.3 m, contains 27,240 kg of sand (particles 0.8-1.2 mm, uniformity coefficient 1.7) and can filter up to 9369 L.min$^{-1}$. The filter is backwashed by water and air and the process is automatically initiated by a clock and when the pressure drop through the filter increases beyond a predetermined level. Manual initiation of the backwash cycle is also possible. Space has been provided for installation of an additional filter to accommodate increases in salmon production. The volume of the head tank is sufficient to provide a continuous flow of water to salmon parr in the aquadomes during the 16-min backwash cycle.

The packed columns reduce the total dissolved gas pressure of the warm water. Each column consists of three, intermittent sections of PVC pipe (each section 25 cm diameter x 46 cm long) filled with 4-cm Koch rings[6]. The Koch rings are designed to have a large surface area and effectively disperse the water so that total gas pressure is reduced. The area is ventilated by louvres and an exhaust fan so that excess nitrogen and oxygen gases are removed from the building. Each column is capable of degassing up to 650 L water.min$^{-1}$. The filtered, warm water plunges into the head tank (3 m x 10 m x 2.3 m deep) and then flows by gravity to the incubation building or to four aquadomes through a 46-cm diameter PVC pipeline. Water which enters the incubation building passes through two ultraviolet water sterilizers[7] and then three interconnected pipe headers, each of which supplies 20 upwelling incubation boxes.

The four aquadomes are prefabricated greenhouses, each measuring 36.6 m x 9.1 m. Translucent fiberglass panels cover the galvanized steel support structures that are installed on concrete foundations. Each aquadome contains 18 3-m fiberglass, Swedish-type[8] tanks. Two of the aquadomes are located on an upper level and two on a lower level so that water from the upper aquadomes can be reused in the lower aquadomes if required. Water delivered to each 3-m tank passes through a packed column composed of three, intermittent sections (each section 15 cm diameter and 46 cm in length) of PVC pipe filled with 4-cm Koch rings. Water drains from each tank to a concrete drainage trench through a perforated aluminum screen (2.4-mm openings) centred in the bottom of the tank. The depth of water in each tank is controlled by a perimeter standpipe connected to the drain sump. The aquadomes are heated by solar energy and ventilated by temperature-controlled louvres and exhaust fans. The temperature of the warm water decreases by about 1°C as it flows from the pump building to the head tank and finally to the incubation building or aquadomes.

---

[6] Koch Engineering Co. Ltd., Calgary, Alberta, Canada.

[7] Aquafine Corporation, Valencia, California, U.S.A.

[8] The tanks measure 3 m x 3 m and have rounded corners.

## BROODSTOCK HOLDING AND SPAWNING

Hatchery-return and wild adult salmon captured in the collection facilities in the Mactaquac Dam during the 15 May-25 October period are transported to sorting facilities at the Mactaquac FCS. Biological information pertaining to the salmon is recorded before some are transferred to broodstock holding ponds at the station and the majority to holding ponds in the sorting facility where they are held until they can be transported by truck to release locations above the Mactaquac Dam. Scales are read to ensure that male, multi-sea-year salmon retained for broodstock purposes have not spawned previously as 1-sea-yr salmon. The majority of female Saint John salmon spend 2 yr at sea before returning to spawn. Egg potential is estimated from the equation:

$$F = 430.19e^{0.03605FL}$$

where F is fecundity and FL is fork length in centimeters (Marshall and Penney 1983).

Three broodstock ponds are utilized to separate the early- and late-run salmon collected at the Mactaquac Dam and salmon collected from tributaries to the river. Two ponds measure 43 m x 4.3 m x 1.4 m deep and the other measures 15 m x 2.5 m x 1.4 m deep. The large ponds each receive 1000 L of well water.min$^{-1}$ (8-9°C) and the small pond half that amount. The broodstock are exposed to a natural photoperiod and males and females are not segregated. Spawning occurs for a 2-wk period during late October and early November. The fertilized eggs are rinsed, allowed to water-harden in aerated well water, and then disinfected by a 10-min immersion in a 100-mg.L$^{-1}$ iodine solution. The eggs are again rinsed, then poured into perforated, rectangular egg trays which are held in fiberglass troughs supplied with aerated well water. The eggs are supplied with well water from 1 November until 25 November when they receive river water which has cooled to 8°C and will continue to cool to 1°C by 15 December.

The holding of salmon broodstock for several months in cool well water which has a constant thermal regime has sometimes resulted in the production of relatively small eggs which have an unacceptably high mortality rate (Farmer, unpubl. data[9]). This problem has been overcome at some locations by exposing the broodstock to surface water which displays a seasonal thermal fluctuation. Egg quality can be determined by measuring the diameter of water-hardened eggs and from mortality rate to the eyed stage. Analysis of covariance was used to adjust the mean diameter of eggs obtained from hatchery-return and wild adult salmon held at Mactaquac in 9°C well water for differences in adult fork length. This adjustment was necessary because egg diameter increases with adult length.

| Origin | Period of capture | Mean fork length, cm | N | Adjusted mean egg diameter, mm | Mean egg mortality, % |
|---|---|---|---|---|---|
| Hatchery-return | 05 Sep-27 Oct | 85.2 | 26 | 6.20 | 7 |
| Wild | 05 Sep-27 Oct | 83.6 | 11 | 5.92 | 6 |
| Hatchery-return | 02 Jun-20 Jul | 80.8 | 72 | 5.81 | 18 |
| Wild | 02 Jun-20 Jul | 82.2 | 36 | 5.91 | 14 |

---

[9] Farmer, G., Department of Fisheries and Oceans, P. O. Box 550, Halifax, N.S. B3J 2S7.

The adjusted mean diameter of the eggs collected from late-run salmon (captured 5 September-27 October) was generally greater than that of the eggs collected from early-run salmon (2 June-20 July) but a significant statistical difference (standard error of difference between adjusted means; $P<0.05$) was only apparent between the hatchery-return, late-run group and the others. Mortality to the eyed stage was observed to be greater among eggs collected from early-run salmon than among eggs from late-run salmon. The duration of adult exposure to 9°C well water at the Mactaquac FCS appears to influence both egg diameter and mortality rate. However, the eggs are considered to be of acceptable quality and broodstock are not held in surface water. The advantage of reduced disease incidence among broodstock held in well water at this location outweighs the improvement in egg quality which may be realized by holding them in warmer surface water which displays a seasonal thermal regime.

## EGG INCUBATION AND ACCELERATED REARING

The growth of fungus on the eggs is controlled by exposure to malachite green (5 $mg.L^{-1}$) for 1 h when required, and by the manual removal of dead eggs. The eggs are usually eyed by 15 December after the accumulation of approximately 240 daily temperature units (°C). They are shocked at that time and the viable eggs surface disinfected using an iodophor. About the middle of January, one million eyed eggs are moved to the Accelerated Rearing Facility and deposited in the upwelling incubation boxes (Fig. 2). Each box contains 10 layers of poultry nesting material[10] which act as a substrate for the eggs. Five thousand eggs are deposited on each piece of poultry nesting material so that there are 50,000 eggs. $box^{-1}$. The pieces of nesting material in each box are separated by pieces of 2.5-cm gravel. Each incubation box receives 23 L of water. $min^{-1}$ which has a temperature of 6°C (Table 1).

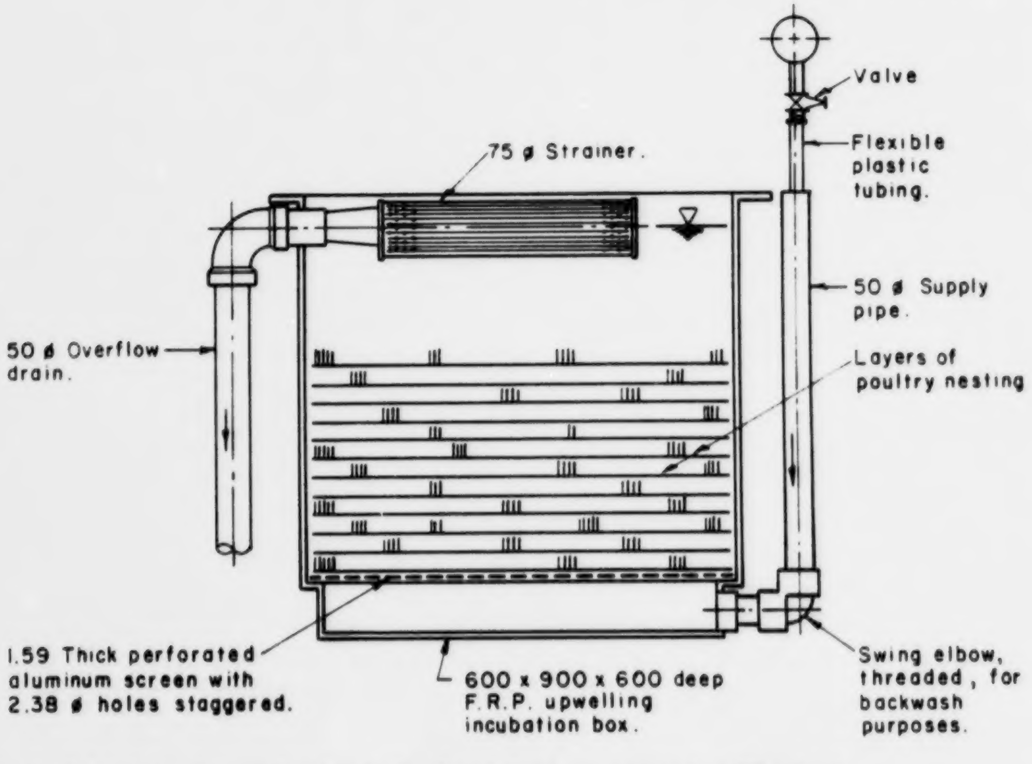

Valve
75 ø Strainer.
Flexible plastic tubing.
50 ø Supply pipe.
50 ø Overflow drain.
Layers of poultry nesting
1.59 Thick perforated aluminum screen with 2.38 ø holes staggered.
600 x 900 x 600 deep F.R.P. upwelling incubation box.
Swing elbow, threaded, for backwash purposes.

**Fig. 2. Schematic of upwelling incubation box (all measurements in millimeters).**

---

[10] Monsanto Plastics and Resins Co., La Salle, Quebec, Canada.

Table 1. Rearing regime at the Mactaquac Accelerated Rearing Facility.

| Date | Stage | Mean weight (g) | Number of rearing units | Salmon. rearing unit$^{-1}$ | Tank density (kg.m$^{-2}$) | Total[a] eggs or salmon | Water temp (°C) | Water flow. unit$^{-1}$ (L.min$^{-1}$) | Total flow (L.min$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 01 Nov[b] | green eggs | - | 18 6-m troughs | 61,100 | - | 1,100,000 | 8 | 23 | 414 |
| 15 Dec | eyed eggs | - | 18 6-m troughs | 56,100 | - | 1,010,000 | 1.5 | 23 | 414 |
| 15 Jan[c] | eyed eggs | - | 20 incubation boxes | 50,000 | - | 1,000,000 | 6 | 22.5 | 450 |
| 10 Feb | alevins | - | 20 incubation boxes | 47,500 | - | 950,000 | 6 | 22.5 | 450 |
| 15 Mar | alevins | - | 20 incubation boxes | 46,250 | - | 925,000 | 8 | 22.5 | 450 |
| 01 Apr | fry | 0.16 | 20 incubation boxes | 45,000 | - | 900,000 | 11 | 22.5 | 450 |
| 05 Apr | fry | 0.16 | 40 3-m tanks | 22,500 | 0.39 | 900,000 | 14 | 32 | 1,280 |
| 19 Apr | fry | 0.25 | 57 3-m tanks | 15,000 | 0.40 | 855,000 | 15 | 45 | 2,565 |
| 03 May | parr | 0.42 | 72 3-m tanks | 10,833 | 0.49 | 780,000 | 15 | 54 | 3,888 |
| 20 May | parr | 0.81 | 72 3-m tanks | 10,556 | 0.92 | 760,000[d] | 15 | 76 | 5,472 |
| 13 Jun[e] | parr | 2.01 | 72 3-m tanks | 10,000 | 2.17 | 720,000 | 15 | 90 | 6,480 |

[a] Decreasing numbers of eggs or salmon represent mean rates of mortality.
[b] Green eggs are incubated at the Mactaquac FCS.
[c] Eyed eggs are transferred to the Accelerated Rearing Facility.
[d] Approximately 40,000 excess parr are removed from the tanks at this time.
[e] Immersion vaccination and transfer of parr to the Mactaquac FCS begins.

The boxes remain covered during January and February to prevent the entry of sunlight and hatching occurs after the eggs have *accumulated about 410 daily temperature units.* Water enters the bottom of each box through a 50-mm diameter supply pipe and flows upward before leaving the box through a 75-mm diameter cylindrical strainer which has 1.2-mm openings. A perforated aluminum screen is mounted in the bottom of each box to disperse the incoming water and prevent the alevins from escaping. Flow can be reversed in the incubators for cleaning purposes by the 90° rotation of the supply pipe after removal of the water supply tubing.

The alevins are provided with water which has a temperature of 6°C until the middle of March when temperature is increased to 8°C. Water temperature is further increased to 11°C during late March and swim-up occurs during the first week of April after the accumulation of 790-820 daily temperature units. The fry are then transferred to the 3-m fiberglass tanks enclosed in the aquadomes. The alevins are relatively inactive while in the upwelling incubation boxes and efficiently utilize their yolk material for growth. This has resulted in *relatively large swim-up fry which have an* average weight of 0.15-0.16 g.

Considerable treatment of the warm water is required so that it is acceptable for salmon alevins and fry. Sand filtration reduces the concentration of total suspended and settleable solids and packed columns are utilized to reduce total dissolved gas pressure. Copper concentration has been measured to ensure that toxic concentrations of this metal are not released from the stator and thrust bearing heat exchangers. Total dissolved gas pressure is dependent upon the temperature increase imparted to the waste warm water and to the amount of cooler water that is mixed with it. Concentrations of suspended and settleable solids increase as the snow melts and runoff peaks during late April and early May. Measurement of these parameters before and after water treatment has enabled an assessment of the effectiveness of the water treatment facilities:

| | | Before treatment | | | After treatment | | |
|---|---|---|---|---|---|---|---|
| Parameter | Year | Mean | Range | N | Mean | Range | N |
| Total dissolved gas pressure, % of atmospheric pressure | 1984 | 114.3 | 106.9-119.0 | 5 | 99.2 | 98.3-99.8 | 5 |
| | 1985 | 110.8 | 108.9-112.5 | 4 | 99.4 | 99.0-100.1 | 5 |
| Total suspended and settleable solids, $mg.L^{-1}$ | 1984 | 6.3 | 0.1-22.5 | 19 | 2.1 | 0.1-6.8 | 20 |
| | 1985 | 4.9 | 0.6-11.7 | 13 | 2.1 | 0.1-5.6 | 13 |
| Copper, $\mu g.L^{-1}$ | 1984 | <10 | all <10 | 7 | No treatment | | |
| | 1985 | <10 | all <10 | 4 | No treatment | | |

The objectives of ensuring that total dissolved gas pressure is not greater than atmospheric pressure, that concentrations of suspended and settleable solids are <10 mg.L$^{-1}$, and that copper concentration is <10 µg.L$^{-1}$ have been attained. Total dissolved gas pressure of the warm water before treatment would result in a significant mortality of alevins and fry and some of the concentrations of suspended and settleable solids measured before sand filtration could cause gill irritation which may result in fungal infection or bacterial gill disease. Concentrations of copper have been <0.1 of the 24-h $LC_{50}$ concentration and are not toxic to salmon.

Fry from each incubation box are transferred to two 3-m fiberglass tanks during early April for preliminary feeding at 14°C (Table 1). Density at that time is about 22,500 fry.tank$^{-1}$ and 40 of the tanks are used. The fry are exposed to a photoperiod of 15 h light and 9 h darkness and provided with food during daylight hours at 1/2-h intervals by hand or by two vibration-type feeders suspended above each tank. Water temperature is increased to 15°C when the fry are observed to be consuming food. They receive an equal mixture of two commercially prepared diets which are fed at the rate of about 5% body weight.day$^{-1}$. One (Biodiet)[11] is an intermediate-moisture diet and the other a dry diet manufactured according to specifications of the Department of Fisheries and Oceans[12]. The intermediate-moisture diet is reported to contain no less than 36% protein and 13.5% lipid and no more than 2% fibre, 10% ash, and 23% moisture. The main ingredients of this diet are fish meal, cooked hydrolyzed fish, and fish oil. The main ingredients of the dry diet are herring or capelin meal and herring, capelin, or salmon oil. The proximate composition of the dry diet is 52% protein, 18% lipid, 2% fibre, <10% ash, and 10% moisture. The proportion of the intermediate-moisture diet in the mixture is gradually reduced after the third or fourth week of feeding until the salmon are receiving only the dry diet. Diet trials have shown that the intermediate-moisture diet is more palatable and results in better survival and growth during the initial few weeks of feeding but that the growth of the salmon is superior when they are provided with the dry diet during subsequent weeks. By providing a mixture of the two diets during the first month, the fry are able to select specific food particles and this appears to enhance their survival and growth. Diet granule size is increased at regular intervals throughout the period of accelerated rearing.

Growth of parr at the facility during the years 1986-88 is described by the following regressions where W is the mean parr weight in grams and D is the total number of days of feeding:

| Year | Regression | N | $R^2$ | Days to attain a weight of 2 g |
|---|---|---|---|---|
| 1986 | $W=0.1513e^{0.0380D}$ | 62 | 0.96 | 68 |
| 1987 | $W=0.1583e^{0.0382D}$ | 25 | 0.99 | 67 |
| 1988 | $W=0.1459e^{0.0371D}$ | 173 | 0.89 | 71 |

From 67-71 d of feeding are required for the parr to attain a mean weight of 2 g (Fig. 3). It has been observed that the parr must be that size by the second week of June if significant numbers are to be 1-yr-old smolts by April-May of the following year. Immersion vaccination[13] of the 720,000 parr occurs during the second week of June, followed by their transfer to the Mactaquac FCS.

During the April-June period when the salmon are reared in the 3-m tanks, the number of parr per tank is decreased at regular intervals so that by late May each tank contains about 10,000 parr. Water flow to each tank is periodically increased during the period to ensure that the tanks are self-cleaning, that fry are dispersed over the entire bottom area and that the water quality within the tanks is of acceptable quality. Water depth within the tanks is maintained at about 20 cm throughout

---

[11] Bioproducts Incorporated, Warrenton, Oregon, U.S.A.

[12] S. Lall, Dept. of Fisheries and Oceans, P.O. Box 550, Halifax, N. S., B3J 2S7, Canada.

[13] Furogen b is manufactured by Aqua Health Ltd., Charlottetown, P.E.I., for the prevention of furunculosis.

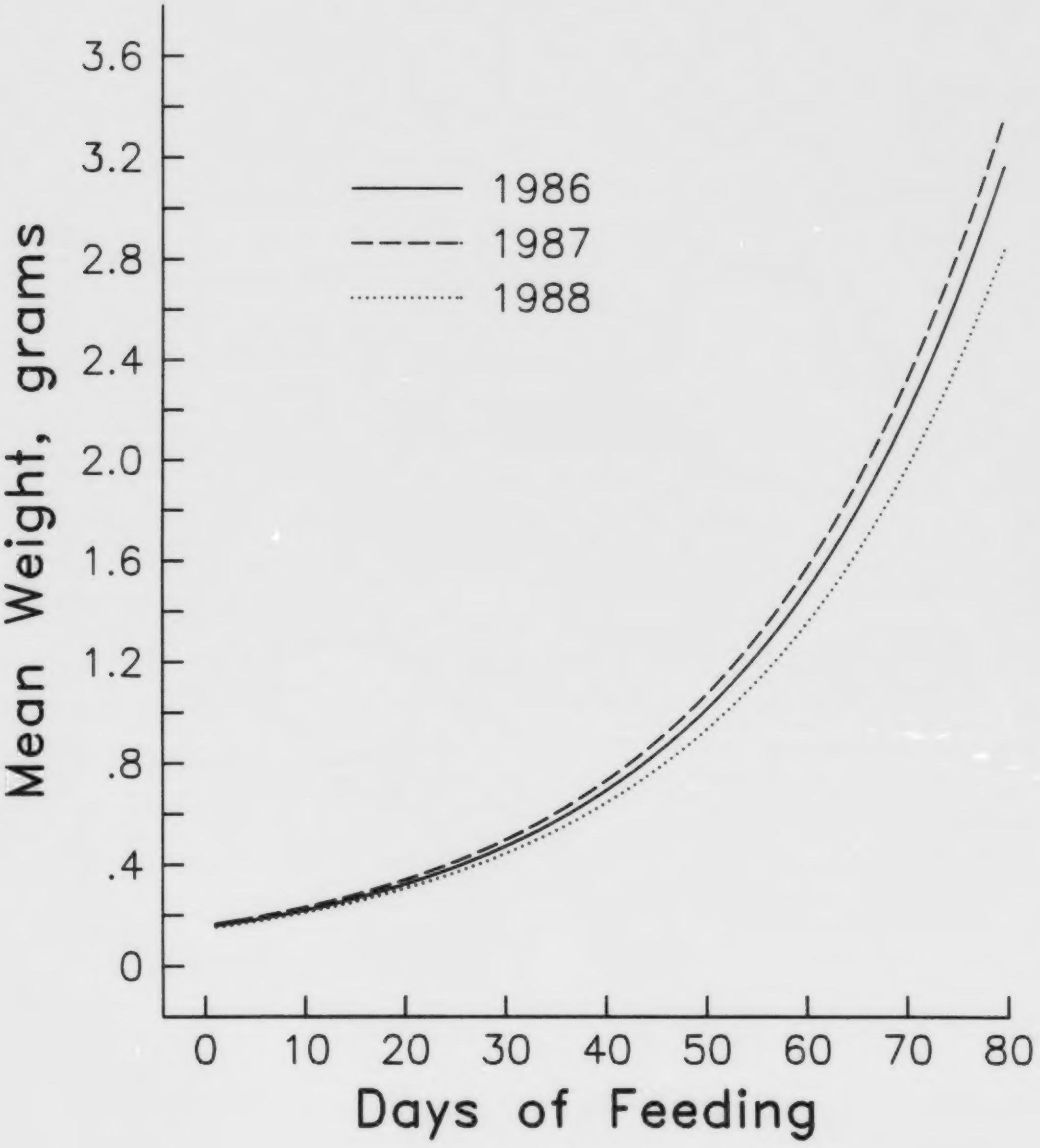


**Fig. 3. Growth of salmon parr at the Accelerated Rearing Facility during the years 1986-88.**

the April-June period. Some chemical characteristics (means) of effluent from the rearing tanks have been: pH 7.22, total suspended and settleable solids 1.7 mg.L$^{-1}$, unionized ammonia 0.001 mg.L$^{-1}$, oxygen 97.3% saturation, and carbon dioxide 2.2 mg.L$^{-1}$, indicating that it is of acceptable quality for reuse when required.

The accelerated rearing regime which has been utilized is best described in Table 1 where the timing of the various developmental stages is shown as well as details of the number of tanks used, the rearing densities, water flows and temperatures, and the growth and survival of the salmon. The use of warm water enables the production of 2-g parr by the second week of June. In contrast, when incubation and early rearing were conducted without the use of the warm waste water, swim-up and preliminary feeding did not occur until early June. Average rates of mortality at the facility have been about 10% among the eyed eggs and alevins contained within the upwelling incubation boxes and 15% during the period the parr are held in the 3-m tanks. During that time, there has been a 12-fold increase in mean weight and an increase in tank density from 0.39 to 2.17 kg.m$^{-2}$. A tank density of 2.17 kg.m$^{-2}$ or 10,000 2-g parr appears to be maximal and growth rate may be reduced if this density is exceeded. Further increases in rearing density may be possible by increasing the 20-cm water depth within the 3-m tanks. Maximum requirement for warm waste water for accelerated rearing occurs during late April and May and corresponds to the period of maximum hydroelectric generation. By late May and early June, the temperature of the river water has increased so that lesser amounts of warm waste water are required. Water demand at the Accelerated Rearing Facility increases from 450 L.min$^{-1}$ in January to about 6480 L.min$^{-1}$ in June.

After transfer of the parr to the Mactaquac FCS, they are reared in 7.6- and 11-m concrete Swedish-type ponds which receive a 16°C mixture of river and ground water. A bimodal length-frequency distribution is evident among the parr by late September when they are graded. The upper growth mode, representing about 55% of the population, is retained and the lower mode released in tributaries of the Saint John River for enhancement purposes. By the following spring, 94% of the upper growth mode are 1-yr-old smolts which have a mean weight of 60 g. The smolts produced at the station are utilized for enhancement of salmon stocks within the Saint John River basin and to assist the New Brunswick salmon aquaculture industry.

## ACKNOWLEDGMENTS

The construction of the Accelerated Rearing Facility was jointly funded by the New Brunswick Electric Power Commission and the Federal Departments of Fisheries and Oceans and Employment and Immigration. The New Brunswick Wildlife Federation sponsored the construction.

## REFERENCES

Marshall, T. L., and G. H. Penny. 1983. Spawning and river escapement requirements for Atlantic salmon of the Saint John River, New Brunswick. Can. Atlant. Fish. Sci. Advis. Comm. Res. Doc. 83/66.

Ruggles, C. P., and W. D. Watt. 1975. Ecological changes due to hydroelectric development on the Saint John River. J. Fish. Res. Board Can. 32: 161-170.

# IMPORTANT CONSIDERATIONS IN THE MARICULTURE OF ARCTIC CHARR (*SALVELINUS ALPINUS* L.)

Julie L. Delabbio, A. Sreedharan[1] and B. D. Glebe[2]
New Brunswick Community College
St. Andrews, New Brunswick E0G 2X0
Canada

[1]Department of Fisheries and Oceans, Biological Station
St. Andrews, New Brunswick E0G 2X0
[2]Huntsman Marine Science Centre, St. Andrews, New Brunswick E0G 2X0

## ABSTRACT

Delabbio, J. L., A. Sreedharan, and B. D. Glebe. 1990. Important considerations in the mariculture of Arctic charr (*Salvelinus alpinus* L.), p. 119-124. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

A number of experiments aimed at assessing the mariculture potential of Arctic charr (*Salvelinus alpinus*) are briefly described and significant findings are discussed. It is shown that charr do not have the same pattern of osmoregulatory response to saltwater as Atlantic salmon. Growth and survival during long-term saltwater rearing is quite variable in Arctic charr. Strain differences and individual differences within strains of Arctic charr are evident in terms of salinity tolerance. Experimentation with charr broodstock indicate that Arctic charr can be held in salt water up until time of spawning. Viable gametes can be obtained from charr broodstock held in salt water.

## RÉSUMÉ

Delabbio, J. L., A. Sreedharan, and B. D. Glebe. 1990. Important considerations in the mariculture of Arctic charr (*Salvelinus alpinus* L.), p. 119-124. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Le présent document décrit brièvement un certain nombre d'expériences qui ont tenté d'évaluer la possibilité d'élever l'omble chevalier (*Salvelinus alpinus*) en milieu marin. On y discute également des principales conclusions de ces expériences. Les expériences démontrent que l'omble chevalier n'a pas le même genre de réaction osmorégulatoire à l'eau salée que le saumon de l'Atlantique. Le taux de croissance et de survie de l'omble chevalier élevé dans l'eau salée pendant longtemps varie énormément. Sa tolérance à la salinité est très différente d'une souche à l'autre et même d'un individu à l'autre à l'intérieur d'une même souche. Des expériences réalisées sur les géniteurs de l'omble chevalier indiquent que ce poisson peut être maintenu dans l'eau salée jusqu'à la période de frai. On peut obtenir des gamètes viables à partir des géniteurs de l'omble chevalier élevés dans l'eau salée.

## INTRODUCTION

In the past few years, the Arctic charr (*Salvelinus alpinus*) has gained prominence as a new candidate species for aquaculture. Initially, it was anticipated that Arctic charr could become a complimentary species of advantage to the established Atlantic salmon seafarming industry. Charr mariculture could provide a diversification of reared species without necessitating the development of new rearing facilities or strategies. This requirement of new facilities and strategies is a prominent problem associated with the development of other marine species for aquaculture.

However, the development of the marine culture of Arctic charr has been hindered because of a supposition that the species has a low salinity tolerance. Early pilot projects in charr mariculture failed (Gjedrem 1975; Sutterlin et al. 1977; Wandsvick and Jobling 1982). It was presumed that this was due in part to a low salinity tolerance that existed throughout the species and which was particularly extreme during low winter water temperatures (Wandsvik and Jobling 1982).

Early research into the mariculture potential of Arctic charr focussed primarily on the performance of only one Norwegian stock. However, it is well known that the Arctic charr is a highly variable species (Balon 1980) and that the species is capable of tolerating extreme environmental conditions (Johnson 1980). Therefore, starting in the spring of 1986, a number of experiments were initiated at the Huntsman Marine Science Centre, using stocks of Canadian Arctic charr, with the purpose of re-examining the mariculture potential of this species. This paper is a summary of some of these experiments and their relevant findings.

## TESTING FOR SALTWATER READINESS

One of the most common research tools used in Atlantic salmon smoltification studies to distinguish whether fish have developed a tolerance for seawater is the 96-h enriched seawater challenge (Saunders and Henderson 1978). In the spring of 1986, this tool was employed in an attempt to distinguish whether 1-yr-old Arctic charr were ready for introduction to seawater. A series of saltwater challenges was run comparing the survival of 2+ Atlantic salmon smolts with several different strains of Canadian Arctic charr. These tests were run from early April to early June. In all challenge tests, except for those involving landlocked charr, the survival rate in the charr strains after 96 h was extremely high (Delabbio, unpubl. data) and comparable to that of the Atlantic salmon. However, it was obvious that some Arctic charr were in a state of severe osmotic stress at the end of the tests. It was found that, in time, these fish would succumb to the stress of exposure to high salinities but that the 96-h test period was not a sufficient period of stress to cause death.

It was concluded that for Arctic charr the 96-h enriched seawater challenge was not a suitable tool for measuring saltwater readiness.

## CHARR OSMOREGULATION IN SALT WATER (Delabbio 1989)

In 1986, a long-term experiment was begun to determine if charr adjust physiologically over time to a change in external osmotic environment (fresh water to salt water). This experiment involved monitoring of the internal osmotic environment of Arctic charr over 9 mo of saltwater rearing. Sixteen-month-old Arctic charr from two anadromous strains were slowly acclimated to salt water. They were then reared in land-based saltwater tanks and sea cages from June 1986-April 1987 at ambient seawater temperatures and natural photoperiods (Fig. 1). Two-yr-old Atlantic salmon (2+ smolts) were reared simultaneously with the charr and under similar conditions. They were used as a comparison or reference group.

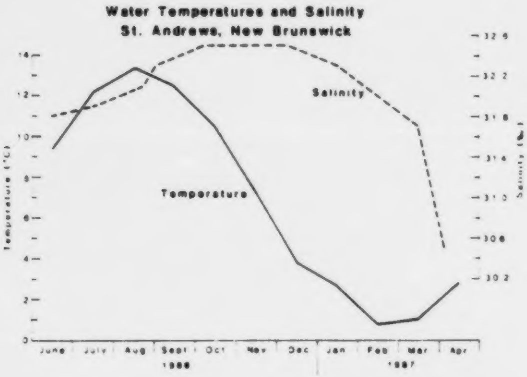


**Fig. 1. Ambient temperature and salinity of salt water for St. Andrews, New Brunswick from June 1986-April 1987.**

Results of this experiment show that throughout the saltwater rearing period, Arctic charr experienced a chronic internal osmotic imbalance. Both plasma osmolality (Fig. 2) and plasma chloride levels increased over time. The differences between Atlantic salmon and Arctic charr in mean values of these two parameters was significant ($P < 0.05$) throughout the study period.

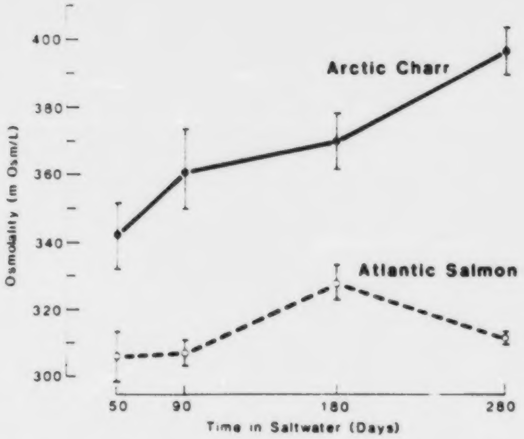


**Fig. 2. Mean plasma osmolality (mOsm/L) of Arctic charr and Atlantic salmon during time in salt water. Values represent ± SEM.**

Examination of gill $Na^+,K^+$-ATPase activity showed that enzyme activity of the Arctic charr was increasing (Fig. 3) and was not significantly different ($P < 0.05$) from that of Atlantic salmon after 180 d in salt water.

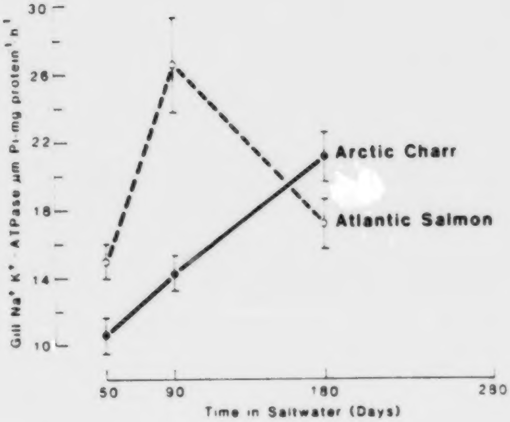


**Fig. 3. Mean gill $Na^+,K^+$-ATPase activity of Arctic charr and Atlantic salmon during time in salt water. Values represent ± SEM.**

## STRAIN DIFFERENCES IN CHARR OSMOREGULATION IN SALT WATER
### (Delabbio 1989)

Two different strains of Arctic charr were used in this long-term physiological study. Examination of the osmoregulatory parameters (osmolality and chloride ion level) of the two different strains showed that there were significant differences in their saltwater osmoregulatory capabilities. Northwest Territories charr had consistently higher mean values in plasma osmolality (Fig. 4) and plasma chloride.

There was, however, no significant difference in mean gill $Na^+,K^+$-ATPase activity between the two charr strains (Fig. 5).

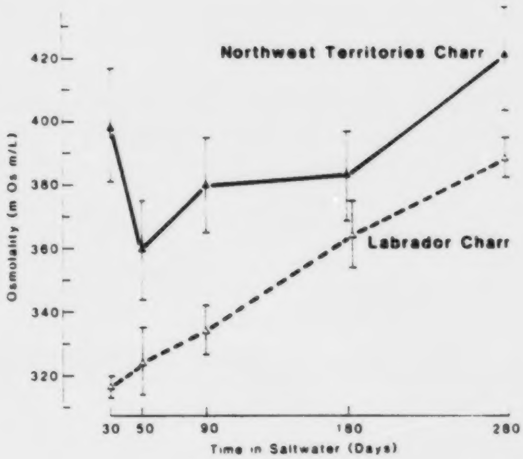


**Fig. 4. Mean plasma osmolality (mOsm/L) of Labrador Arctic charr and Northwest Territories Arctic charr during time in salt water. Values represent ± SEM.**

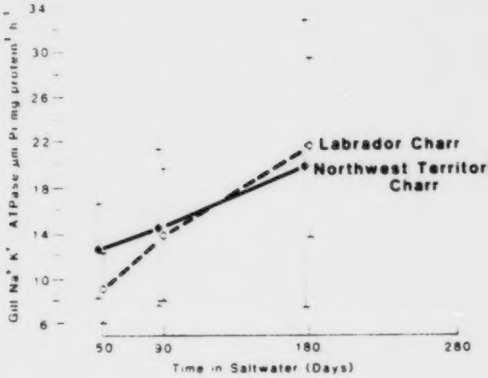


**Fig. 5. Mean gill $Na^+,K^+$-ATPase activity of Labrador Arctic charr and Northwest Territories Arctic charr during time in salt water. Values represent ± SEM.**

## STRAIN DIFFERENCES IN SURVIVAL AND GROWTH IN SALT WATER

A saltwater growth study involving two charr strains and Atlantic salmon was initiated in August 1986 and extended to April 1987 (Delabbio et al. 1990).

Fish from both strains of Arctic charr were able to survive Canadian winter seawater temperatures as low as 0.05°C (Fig. 1). Mortality among both charr strains was, however, higher than that exhibited by the Atlantic salmon.

The body size of the Arctic charr mortalities suggest that a "threshold" body weight for successful saltwater introduction exists in the species. Over 90% of charr mortalities were below 200 g body weight (Fig. 6).

In the Labrador Arctic charr strain, a bimodal distribution in weight frequency classes developed during the 9-mo grow-out period. Of particular note was the fact that the charr in the upper modal group had achieved a mean body weight 35% greater than the mean Atlantic salmon body weight by the end of the study period.

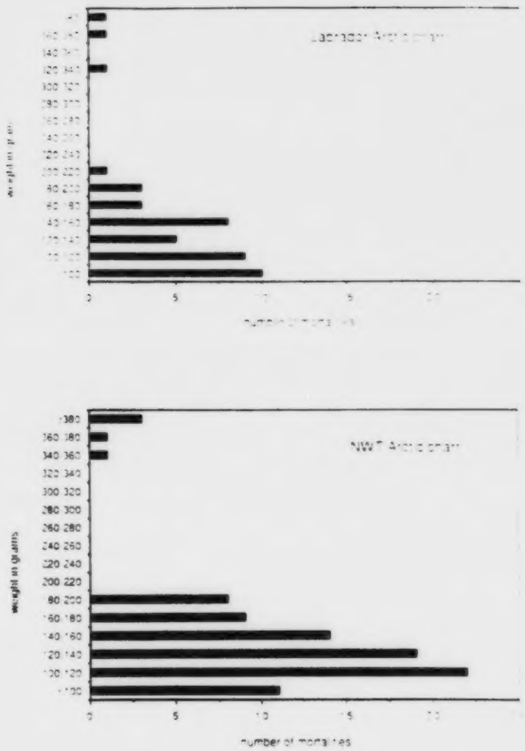


**Fig. 6. Weight distribution of mortalities of Labrador Arctic charr and Northwest Arctic charr during time in salt water.**

## SALINITY REQUIREMENTS FOR HOLDING BROODFISH

In Atlantic Canada, most Atlantic salmon broodstock are maintained in, and spawned directly from, salt water. To determine if this procedure could be applied to Arctic charr broodstock, charr were reared under three different salinity regimes prior to spawning (Delabbio et al. 1989). In the first treatment group, charr broodfish were reared for 32 mo in fresh water. In the second group, charr broodfish were held for 16 mo (posthatch) in fresh water, then acclimated to salt water (32 ppt salinity) and reared for 14 mo, and then returned to fresh water 2 mo prior to spawning. In the third treatment group, Arctic charr were held 21 mo in fresh water and then reared for 11 mo in salt water up until time of spawning.

At spawning time, both groups of fish held in fresh water had lower blood plasma osmolalities and chloride ion levels than broodstock that remained in salt water. Broodstock osmoregulatory status did not seem to affect gamete quality directly. Eggs from saltwater broodfish had the highest percent eye-up, while eggs taken from fish that had been transferred to salt water 2 mo prior to spawning had the lowest percent eye-up (Table 1).

The experiment indicated that charr broodstock could be maintained in salt water up to time of spawning and still produce viable gametes.

## GENERAL DISCUSSION

The Arctic charr has been described as one of the salmonids displaying the lowest degree of anadromy (Hoar 1976). However, this does not necessarily mean that the fish has the least amount of salinity tolerance. Anadromous behavior must be seen as somewhat independent on environmental conditions. Therefore, environmental constraints such as lethal low winter water temperatures may be the factor compelling all oceanic charr to move into fresh water -- not decreasing salinity tolerance in colder water as previous studies had presumed. This is supported by the results in our growth experiment where Arctic charr were able to overwinter in salt water.

During the extended saltwater growth studies, the charr mortality exceeded acceptable commercial standards for Atlantic salmon. However, it must be remembered that, just as in the early days of Atlantic salmon seafarming, knowledge about the environmental and biological requirements for saltwater rearing of this new aquaculture species is quite limited. Since some Arctic charr not only survived, but also grew during extended saltwater holding, it is suggested that the saltwater culture of Arctic charr is feasible but further research must be done to raise rearing success to acceptable commercial levels.

Table 1. Mean egg fecundity, mean egg size, and percent egg eye-up from Arctic charr broodstock held in three different salinity treatments.

| | Broodstock treatment | | |
|---|---|---|---|
| | Fresh water | Mixed | Salt water |
| $\bar{X}$ fecundity (no. eggs/female) | 2178.0 | 1550.0 | 1431.0* |
| $\bar{X}$ eggs/g fish | 3.4 | 2.9 | 2.3* |
| $\bar{X}$ egg size (mm) | 3.9 | 3.5 | 3.6* |
| % eye-up | 41.1 (.695) | 19.7 (.387) | 4.6 (.175) |

*No significant difference between treatments at $P > 0.05$.
() Represent arc-sine transformation values.

Similarly, the limited success in spawning Arctic charr directly from salt water should not be seen as a deterrant to the mariculture of the species. This investigation indicated that it is possible to spawn Arctic charr directly from salt water. Improvements on this technique are now required.

In general, from the results obtained in these experiments, it is clear that the pattern of saltwater adaptation in charr is different from that in Atlantic salmon.

It is, however, apparent from these experimental results that very little is understood about the saltwater tolerance of this most northern dwelling of anadromous salmonids. For example, how do some individual charr withstand high internal osmotic imbalance and continue to survive and grow?

Arctic charr is a highly adaptive, extremely diverse species. These studies indicate that some of the problems that were previously considered as severely limiting to the mariculture potential of the species may be solved through continued research.

## REFERENCES

Balon, E. K. 1980. Preface. In E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands, 98 p.

Delabbio, J. L. 1989. A comparative study of two anadromous strains of Canadian Arctic charr (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) in a saltwater environment. M.Sc. Thesis, University of New Brunswick, Fredericton, 114 p.

Delabbio, J. L., M. Sweeney, and B. D. Glebe. 1989. Maturation and spawning of Arctic charr (*Salvelinus alpinus*) at different salinities. In H. Kawanabe, F. Yamazaki and D. L. Noakes (ed.) Biology of charrs and masu salmon. Physiology and Ecology Japan Special Vol. 1, 1989: 504.

Delabbio, J. L., B. D. Glebe, and A. Sreedharan. 1990. Variation in growth and survival between two anadromous strains of Canadian Arctic charr (*Salvelinus alpinus*) during long-term saltwater rearing. Aquaculture 85: 259-270.

Gjedrem, T. 1975. Survival of Arctic charr in the sea during fall and winter. Aquaculture 6: 189-190.

Hoar, W. S. 1976. Smolt transformation: evolution, behaviour and physiology. J. Fish. Res. Board Can. 33: 1233-1252.

Johnson, L. 1980. The Arctic charr, *Salvelinus alpinus*. In E. K. Balon (ed.) Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands, 98 p.

Saunders, R. L., and E. B. Henderson. 1978. Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 1542-1546.

Sutterlin, A. M., L. R. MacFarlane, and P. Harmon. 1977. Growth and salinity tolerance in hybrids within *Salmo* spp. and *Salvelinus* spp. Aquaculture 12: 41-52.

Wandsvik, A., and M. Jobling. 1982. Overwintering mortality of migratory Arctic charr, *Salvelinus alpinus* (L.), reared in salt water. J. Fish Biol. 20: 701-706.

# THE ROLE OF GROWTH HORMONE IN THE ADAPTABILITY OF ATLANTIC SALMON (*SALMO SALAR*) TO SEAWATER

G. Boeuf, A. Le Roux, A. Severe, P. Prunet[1] and P. Y. Le Bail[1]
IFREMER, Centre de Brest
BP 70, 29280 Plouzane
France

[1] INRA, Physiologie des poissons, campus de Beaulieu,
35042 Rennes cedex, France

## ABSTRACT

Boeuf, G., A. Le Roux, A. Severe, P. Prunet, and P. Y. Le Bail. 1990. The role of growth hormone in the adaptability of Atlantic salmon (*Salmo salar*) to seawater, p. 125-131. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Gill $Na^+,K^+$-ATPase activity of juvenile Atlantic salmon reared in fresh water during three successive years increased from February to April-May, while blood plasma GH levels increased strongly from the beginning of April and thereafter remained high. Direct transfer to full salinity seawater triggered a transitory increase in growth hormone (GH) at any time of year. A cholesterol implant containing ovine growth hormone (oGH) in presmolts (5-6 mo before migration of 0-age fish stimulated gill $Na^+,K^+$-ATPase activity, resulted in high survival in full salinity (35 o/oo) seawater, good osmotic regulation, and subsequent good growth. Implantations of oGH in small parr resulted in 36% survival after 8 wk in seawater, compared with 0% in controls. Possible roles of GH are discussed during parr-smolt transformation.

## RÉSUMÉ

Boeuf, G., A. Le Roux, A. Severe, P. Prunet, and P. Y. Le Bail. 1990. The role of growth hormone in the adaptability of Atlantic salmon (*Salmo salar*) to seawater, p. 125-131. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'activité $Na^+,K^+$-ATPasique branchiale de juvéniles de saumon atlantique maintenus en eau douce augmente régulièrement de février à avril-mai durant trois années successives tandis que le niveau circulant d'hormone de croissance (GH) s'élève plus ou moins brusquement à partir de début avril et ensuite se maintient à valeur plus forte. Des transferts directs à pleine salinité (35 o/oo) déclenchent une augmentation transitoire de la GH quelque soit le moment de l'année. Une implantation de GH ovine contenue dans de la poudre de cholestérol compactée a permis chez des pré-smolts de lère année (à plus de 5-6 mois de la migration) de stimuler l'activité ATPasique branchiale en eau douce, une excellente survie en eau salée (35 o/oo) après transfert, une bonne osmorégulation et une bonne croissance par la suite. L'implantation de petits parrs avec de la oGH au même moment nous a donné une survie de 36% après 8 semaines en mer comparativement à 0% pour les témoins. Nous discutons les implications possibles de la GH dans la smoltification.

## INTRODUCTION

The physiological, biochemical, and endocrinological changes occurring at smolting have been reviewed by Fontaine (1975), Wedemeyer et al. (1980), Boeuf (1987), and Hoar (1988). Smolts are able to tolerate direct transfer to seawater (35 o/oo) without osmotic disequilibrium, and continue to grow (Parry 1960; Boeuf 1987; Boeuf et al. 1989a). In contrast, juvenile salmon which have either lost or not attained smolt status are unable to adapt, survive, or grow in full strength seawater. Among the hormones involved in the parr-smolt transformation, growth hormone (GH) seems to play a major role. Treatments of fish with mammalian GH demonstrated a possible influence of this hormone on growth (Donaldson et al. 1979), smoltification, and seawater adaptation (Komourdjian et al. 1976; Clarke et al. 1977; Nagahama et al. 1982). With the advent of salmon homologous GH RIAs, Sweeting et al. (1985), Young et al. (1989) for coho salmon (*Oncorhynchus kisutch*), then Boeuf et al. (1989a) and Prunet et al. (1989) for Atlantic salmon (*Salmo salar*), demonstrated the occurrence of a GH surge at the end of smolting of these species in fresh water. After transfer of salmonids from fresh water to seawater, GH increases sharply some hours after transfer (Bolton et al. 1986; Boeuf et al. 1989a).

We studied the changes in circulating concentration of GH in juvenile salmon during smolting and after transfer to seawater, and also tested the effects of GH implants on seawater adaptability and subsequent growth of potential smolts and parr.

## MATERIALS AND METHODS

Fish used in the experiments were of a Norwegian strain of cultured salmon (1986, 1987, and 1988) introduced to the Le Conquet hatchery (Brittany, 48° N). Elorn River fish (natural population of Brittany) were used for the implant studies. The fish were reared in fresh water in Ewos tanks (4 $m^2$) under natural photoperiod and temperature. River pH was 6.5-7.0 and temperature changes from 10°C in December to 5°C in February and 15°C in June (see Boeuf et al. 1989a, b for details). From September preceding the smolting year (7 mo old), upper modal group fish (Boeuf et al. 1985) were selected for experiments and maintained at a stocking density of 100/$m^2$ and fed automatically using dry pellets (SS1 IFREMER). The seawater transfers involved transporting the fish to the Centre Océanologique de Bretagne in Brest, at least 2 wk before, where they were held in freshwater tanks identical to those used later with seawater. On the day of transfer, the freshwater (FW) supply was stopped, the seawater (SW) supply started, and the fish were in full seawater (34.5-35.6 o/oo) within 1 h.

Gill and plasma samples (from 10-25 fish) were taken both in FW and SW according to the methods described in Boeuf et al. (1985, 1989a). Gill $Na^+,K^+$-ATPase activity and blood osmotic pressure were measured as described in Lasserre et al. (1978) and Boeuf et al. (1989a). Plasma GH levels were measured according to the RIA described by Le Bail et al. (in press).

For the GH implant experiments, salmon were selected in the upper mode (presmolts, 129 mm) of the population for the October experiment, and both lower (parr, 89 mm) and upper (presmolts, 134 mm) modes in November. In each experiment, we used three groups of 50 salmon held in the same tank, either untreated (control), or intraperitoneally implanted (Higgs et al. 1975) with a compacted cholesterol powder pellet (sham) or with 250 µg of ovine GH (NIADK-oGH-14) in the same type of pellet. Each group was identified using the scar of the implantation and the presence or absence of adipose fin. Each pellet weighed 8-10 mg and released GH during 3-4 wk (Le Bail, unpubl. data). Fish were transferred to seawater, as previously described, after 11-12 d.

One-way ANOVA, SNK multiple range and Student's t-tests were used to assess the statistical significance in the data.

## RESULTS

### FRESH WATER

In each of the 3 yr of the study, gill microsomal $Na^+,K^+$-ATPase activity rose gradually from February (average length, 130-140 mm) to mid-April (140-165 mm), mid-May (160-190 mm) ($P < 0.01$-$0.01$), and then decreased. Blood plasma GH was very low in February-March, then increased sharply from the beginning of April, and remained at a high level during May ($P < 0.001$). In 1987, an acute surge occurred in April ($P < 0.001$). In each year of the study, plasma GH remained at higher mean levels after the peaks than before (Fig. 1).

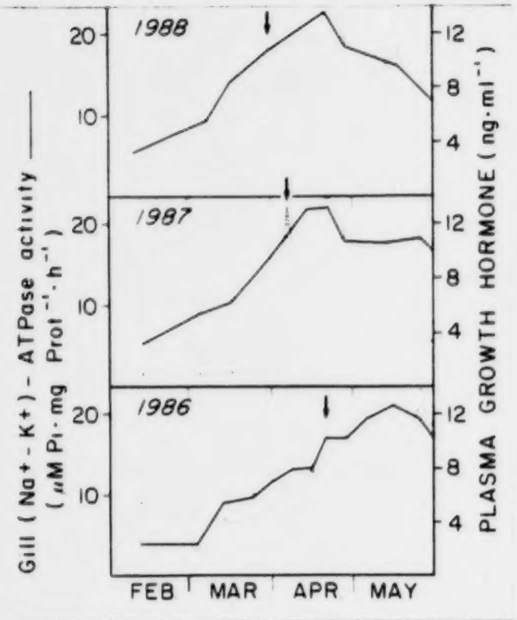


**Fig. 1.** Gill Na$^+$,K$^+$-ATPase activity and GH changes during the smolting period in freshwater Atlantic salmon reared in the hatchery of Le Conquet in 1986, 1987 and 1988. Data are mean ± standard error of the mean for 10-25 fish. The arrows indicate the times of $T_4$ surges (see Boeuf and Prunet 1985; Prunet et al. 1989; Boeuf et al. 1989a, b).

## SEAWATER

After seawater transfers in presmolting (March), smolting (April), and postsmolting periods (May), plasma GH increased significantly ($P < 0.001$-$0.01$) and remained at a high level during 7 d, compared with that in fresh water. After 14 d, there were no significant differences between plasma GH levels in the fish transferred to seawater and freshwater controls (Fig. 2). New results obtained in 1989 confirmed these changes.

## IMPLANTATIONS

In each experiment, the control fish either did not survive transfer to seawater or, if they survived, they did not grow well. In contrast, GH implanted presmolts showed good adaptation to seawater, survival, and growth. Gill Na$^+$,K$^+$-ATPase activity doubled in 11-12 d ($P < 0.01$-$0.001$) compared with controls. GH implanted fish maintained normal osmotic pressure. Implanted parr had much better survival than control fish and had good growth.

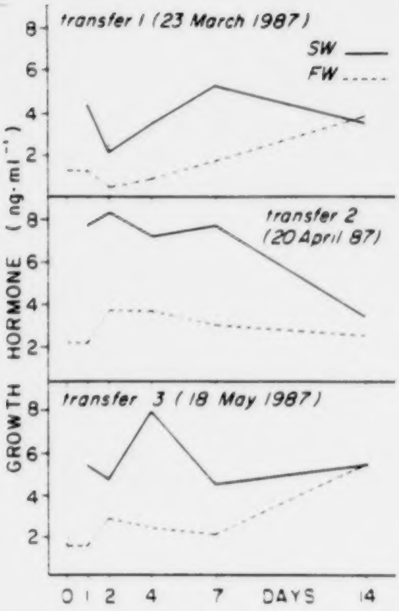


**Fig. 2.** Changes in blood plasma growth hormone levels after direct transfer of presmolts, smolts, and postsmolts of Atlantic salmon from fresh water to seawater at three dates. Data points show mean ± standard error of the mean for 10 fish.

For both parts of the population (implanted parr and presmolts), no mortality occurred between 21 and 54 d in seawater.

## DISCUSSION

Few studies have been published on the role of GH during salmonid smoltification (Sweeting et al. 1985; Young et al. 1989; Boeuf et al. 1989a). In Atlantic salmon completing smoltification, GH peaks occur coincidentally with increases in $T_3$ (Boeuf et al. 1989a; Prunet et al. 1989). In fish in short rivers, the first GH increase appears before, during, or after the $T_4$ surge, 2-3 wk before the maximum gill ATPase level. In comparison, salmon from a long river (Loire-Allier in France, more than 1000 km), showed an increase of plasma GH much earlier in the year (February) and remained high until April (Boeuf et al., unpubl. data). These results suggest that in addition to having a role in osmoregulation, GH may also be involved in migration behavior.

Donaldson et al. (1979) reviewed the clear effects of mammalian GH on growth of salmonids and fish in general and, in addition, this hormone is also very active during smolting, an intense growth phase in salmonid life. In addition to its growth stimulating effect, GH appears to play other roles during smolting: following transfer to seawater, there are significant increases in plasma GH levels, suggesting that this hormone is involved in osmoregulation. These increases are independent of season. GH injections or implants (Richman and Zaugg 1987) (Table 1, 2) are more effective than thyroid hormone (TH) treatments (by injections, in the food, in water...) (Miwa and Inui 1985; Saunders et al. 1985; Boeuf, unpubl. data) in stimulating gill ATPase activity, and improving seawater adaptability (Komourdjian et al. 1976; Miwa and Inui 1985). Miwa and Inui (1985) specified that GH and $T_3$ work synergistically to promote adaptation to seawater. GH is also very active on peripheral deiodination of $T_4$ to $T_3$ (De Luze and Leloup 1984) and it may be that this phenomenon is of fundamental importance during parr-smolt transformation.

In the November experiment (Table 2), the effects of oGH implantation are very clear; treated fish showed a stimulation of gill ATPase activity, control of osmotic disequilibrium after direct full salinity seawater contact, and grew well in seawater. The idea of using GH treatment to stimulate seawater adaptability is not new but previous studies required long series of injections which were very stressful and did not allow the fish to feed normally. However, by injections of GH, Komourdjian et al. (1976) and Clarke et al. (1977) obtained a better adaptation to low salinity seawater (29 or 30 o/oo) than in controls or sham injected salmon. More recently, bGH implants stimu-

Table 1. Results (mean ± standard error of the mean for eight fish) of three batches of Atlantic salmon presmolts after direct transfer to seawater (35 o/oo), 12 d after implantation (or not) with ovine growth hormone (oGH) on October 26 (E1). Weight and fork length are expressed in g and mm, plasma osmotic pressure in milliosmoles per liter and gill ATPase activity in micromoles of inorganic phosphate·mg of protein$^{-1}$·h$^{-1}$ of incubation at 37°C. Fish were directly transferred to seawater (35 o/oo, 12°C) on November 7, 1988. Implants were of powdered cholesterol with (experimental) or without (sham) oGH.

| | | No manipulation (control) | Implanted "sham" | Implanted oGH (250 µg) |
|---|---|---|---|---|
| Starting FW | Weight | 28.3±0.8 | 27.7±0.8 | 26.8±0.7 |
| Oct. 26 1988 | Length | 130±1 | 129±1 | 129±1 |
| Day of transfer | Gill $Na^+,K^+$-ATPase activity | 3.1±0.5 | 3.1±0.2 | 6.4±0.4 |
| 48 h seawater | Mortality | 35% | 20% | 0% |
| 21 d seawater | Mortality | 75% | 50% | 0% |
| | Weight | 27.1±2.2<br>-4.3% | 27.9±1.3<br>+0.72% | 34.0±1.6<br>+26.9% |
| | Length | 133±3<br>+2.3% | 133±2<br>+3.1% | 143±2<br>+10.9% |
| | Osmotic pressure | 345±6 | 341±4 | 340±4 |
| | Gill ATPase activity | 14.2±1.8 | 17.7±1.5 | 20.4±1.0 |

Table 2. Results (mean ± standard error of the mean for eight fish) of three batches of Atlantic salmon presmolts and parr implanted (or not) on November 24 (E2). Fish were directly transferred to seawater (35 o/oo, 12°C) on December 5, 1988. Implants were of powdered cholesterol with (experiment) or without (sham) oGH.

| | | No manipulation (control) | Implanted "sham" | Implanted oGH (250 μg) |
|---|---|---|---|---|
| Starting<br>FW 12°C<br>Nov. 24, 1988 | Weight of presmolts | 30.9±0.4 | 30.8±0.4 | 30.2±0.3 |
| | Length of presmolts | 134±0.4 | 135±0.5 | 134±0.4 |
| | Weight of parr | 8.1±0.2 | 7.9±0.2 | 7.9±0.2 |
| | Length of parr | 89±0.7 | 88±0.5 | 89±0.7 |
| Day of transfer presmolts | Gill $Na^+$,$K^+$-ATPase activity | 3.6±0.7 | 4.1±0.6 | 7.1±0.9 |
| 48 h in seawater | Mortality | 24% | 8% | 0% |
| | Osmotic pressure | 427±10 | 405±18 | 338±6 |
| 7 d in seawater | Osmotic pressure | 423±8 | 383±10 | 326±3 |
| 21 d in seawater | Mortality | 100% | 93% | 6% |
| | Weight | - | 29.5±1.5<br>-4.2% | 34.0±0.6<br>+12.6% |
| | Length | - | 134±2<br>-0.7% | 147±1<br>+9.7% |
| | Osmotic pressure | - | 343±8 | 318±4 |
| | ATPase activity | - | 19.4±7.9 | 25.0±1.0 |
| 54 d in seawater | Weight | - | - | 47.4±1.6<br>+56.9% |
| | Length | - | - | 158±1.7<br>+17.9% |
| Parr | Mortality 48 h | 100% | 88% | 48% |
| 21 d in seawater | Mortality | 100% | 100% | 64% |
| | Weight | - | - | 11.1±1.1<br>+40.5% |
| | Length | - | - | 100±3<br>+12.4% |
| 54 d in seawater | Weight | - | - | 20.2±2.7<br>+155.7% |
| | Length | - | - | 119±6.1<br>+33.7% |

lated growth (Down et al. 1988) and increased gill ATPase activity (Richman and Zaugg 1987) but did not promote seawater adaptability. From our results, presmolts and parr responded to GH and adapted to high salinity (35 o/oo). Transfer of control fish to salt water, as already described (Parry 1960; Boeuf et al. 1989a), showed either high mortalities or poor growth.

In conclusion, oGH implants appear to trigger reactions in the fish which would normally develop only 5-6 mo later during smolting. They caused development of smolt characteristics, including stimulation of the gill ATPase activity, salinity tolerance, osmotic adaptation, and growth in high salinity. From the results, we cannot exclude a slight role of the cholesterol powder, this compound serving as, perhaps, a precursor of corticosteroids, which are also active in seawater adaptability (Hoar 1988).

These data show that GH plays a major role in the parr-smolt transformation phenomenon and seawater adaptation. Smolting salmonids develop, in fresh water, most of the systems they need for survival and growth in salt water; migration or seawater transfer may act only as a final stimulus to complete these processes. GH appears to facilitate some of these transformations, not only in terms of growth, but also in several changes occurring at this time.

## ACKNOWLEDGMENTS

We thank B. Petton and Y. Normant for their excellent technical assistance and J. L. Gaignon for providing the fish. We also thank our colleagues, Gibassier and Chevanne, from the Faculty of Pharmacy in Rennes who made the implants. We gratefully thank the National Hormone and Pituitary Program in Baltimore who gave us the oGH. We also thank R. L. Saunders, G. Farmer and M. Sinclair for their kind invitation and financial help to travel to St. Andrews and Halifax.

## REFERENCES

Boeuf, G. 1987. Contribution à l'étude de l'adaptation à l'eau de mer chez les poissons salmonidés - détermination de critères de smoltification par mesures de l'activité (Na$^+$K$^+$)-ATPasique des microsomes de la branchie et des hormones thyroidiennes plasmatiques. Thèse de Doctorat d'Etat, Université de Bretagne Occidentale, 370 p.

Boeuf, G., A. Le Roux, and Y. Harache. 1985. Gill (Na$^+$K$^+$)ATPase activity and smolting in Atlantic salmon (*Salmo salar* L.) in France. Aquaculture 45: 73-81.

Boeuf, G., P. Y. Le Bail, and P. Prunet. 1989a. Growth hormone and thyroid hormones during Atlantic salmon, *Salmo salar* L., smolting and after transfer to seawater. Aquaculture 82: 257-268.

Boeuf, G., L. M. Uin, and J. G. Eales. 1989b. Plasma levels of free and bound thyroid hormones during parr-smolt transformation in Atlantic salmon, *Salmo salar* L. Can. J. Zool. 67: 1654-1658.

Boeuf, G., and P. Prunet. 1985. Measurements of gill (Na-K)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon, *Salmo salar* L. Aquaculture 45: 111-119.

Bolton, J., N. L. Collie, M. Kawauchi, and T. Hirano. 1986. Osmoregulatory actions of growth hormone in rainbow trout (*Salmo gairdneri*). J. Endocrinol. 112: 63-68.

Clarke, W. C., S. W. Farmer, and K. M. Hartwell. 1977. Effect of teleost pituitary growth hormone on growth of *Tilapia mossambica* and on growth and seawater adaptation of sockeye salmon (*O. nerka*). Gen. Comp. Endocrinol. 33: 174-178.

De Luze, A., and J. Leloup. 1984. Fish growth hormone enhances peripheral conversion of thyroxine to triiodothyronine in the eel, *Anguilla anguilla* L. Gen. Comp. Endocrinol. 56: 308-312.

Donaldson, E. M., U. H. M. Fagerlund, D. A. Higgs, and J. R. McBride. 1979. Hormonal enhancement of growth, p. 456-498. In W. S. Hoar, D. J. Randall and R. Brett (ed.) Fish Physiology, Vol. 8. Academic Press, New York, NY.

Down, N. E., E. M. Donaldson, H. M. Dye, K. Langley, and L. M. Souza. 1988. Recombinant bovine somatotropin more than doubles the growth rate of coho salmon (*Oncorhynchus kisutch*) acclimated to seawater at ambient winter conditions. Aquaculture 68: 141-155.

Fontaine, M. 1975. Physiological mechanisms in the migration of marine and amphihaline fish. Adv. Mar. Biol. 13: 241-355.

Higgs, D. A., E. M. Donaldson, H. M. Dye, and J. R. McBride. 1975. A preliminary investigation of the effect of bovine growth hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 27: 240-253.

Hoar, W. S. 1988. The physiology of smolting salmonids, p. 275-343. In W. S. Hoar and D. J. Randall (ed.) Fish Physiology, Vol. XI,B. Academic Press, New York, NY.

Komourdjian, M. P., R. L. Saunders, and J. C. Fenwick. 1976. The effect of porcine somatotropin on growth and survival in seawater of Atlantic salmon, *Salmo salar*, parr. Can. J. Zool. 54: 531-535.

Lasserre, P., G. Boeuf, and Y. Harache. 1978. Osmotic adaptation of *Oncorhynchus kisutch* Walbaum. I. Seasonal variations of gill $Na^+,K^+$-ATPase activity in salmon, 0+-age and yearling, reared in fresh water. Aquaculture 14: 365-382.

Le Bail, P. Y., J. Carragher, and J. Sumpter. 1991. Development and validation of a chinook GH radio-immunoassay. Gen. Comp. Endocrinol. (in press).

Miwa, S., and Y. Inui. 1985. Effects of L. thyroxine and ovine growth hormone on smoltification of amago salmon *Oncorhynchus rhodurus*. Gen. Comp. Endocrinol. 58: 436-442.

Nagahama, Y., S. Adachi, F. Tashiro, and E. G. Grau. 1982. Some endocrine factors affecting the development of seawater tolerance during the parr-smolt transformation of the amago salmon *Oncorhynchus rhodurus*. Aquaculture 28: 81-90.

Parry, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar*, and some related species. J. Exp. Biol. 37: 425-434.

Prunet, P., G. Boeuf, J. B. Bolton, and G. Young. 1989. Smoltificaiton and seawater adaptation in Atlantic salmon (*Salmo salar* L.): plasma prolactin, growth hormone and thyroid hormones. Gen. Comp. Endocrinol. 74: 355-364.

Richman, N. H., and W. S. Zaugg. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and post-smoltified coho salmon *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 65: 189-198.

Saunders, R. L., S. D. McCormick, E. B. Henderson, J. G. Eales, and C. E. Johnston. 1985. The effect of orally administered 3,5,3'triiodo-L-thyronine ($T_3$) on growth and salinity tolerance of Atlantic salmon *Salmo salar* L. Aquaculture 45: 143-156.

Sweeting, R. M., G. F. Wagner, and B. A. McKeown. 1985. Changes in plasma glucose, amino acid nitrogen and growth hormone during smoltification and seawater adaptation in coho salmon *Oncorhynchus kisutch*. Aquaculture 45: 185-197.

Wedemeyer, G., R. L. Saunders, and W. C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1-14.

Young, G., B. T. Bjornsson, P. Prunet., R. J. Lin, and H. A. Bern. 1989. Smoltificaiton and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones and cortisol. Gen. Comp. Endocrinol. 74: 335-345.

# SALMONID GROWTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS: TOWARD A GENERAL GROWTH MODEL FOR CHINOOK SALMON

Henrik Kreiberg
Department of Fisheries and Oceans
Pacific Biological Station
Nanaimo, British Columbia V9R 5K6
Canada

## ABSTRACT

Kreiberg, H. 1990. Salmonid growth under different environmental conditions: toward a growth model for chinook salmon, p. 133-136. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

An experimental transformation was applied to daily water temperature data sets from a comparative study of growth of chinook salmon in sea-cage culture in B.C. Use of the transformation reduced, but did not eliminate, the effect of different thermal histories among rearing sites. It was not possible to propose a general model for chinook growth in cage culture using this particular compensation for inter-site differences in temperature. The transformation suggested that an optimum for growth might lie in the range of 13-14°C.

## RÉSUMÉ

Kreiberg, H. 1990. Salmonid growth under different environmental conditions: toward a growth model for chinook salmon, p. 133-136. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Une transformation expérimentale a été appliquée à des séries de données sur la température quotidienne de l'eau, données qui ont été tirées d'une étude comparative de la croissance du saumon quinnat dans des cages marines submergées en Colombie-Britannique. Cette transformation a réduit, mais non éliminé, l'effet des différents antécédents thermiques parmi les divers lieux d'élevage. Il n'a pas été possible de proposer un modèle général pour étudier la croissance du saumon quinnat en utilisant cette façon particulière de compensation des écarts de températures entre les divers sites. La transformation laisse supposer que la meilleure température pour favoriser la croissance serait de 13 à 14°C.

## INTRODUCTION

The chinook salmon (*Oncorhynchus tshawytscha*) has been cultured in sea cages in B.C. since the early 1970s. Since the mid-1980s, simultaneous developments in nutritional knowledge and the expansion of a commercial culture industry based on a 2- to 5-kg fresh-market fish have resulted in significant culture activity. It is expected that B.C.'s production of chinook in 1989 will reach 13,000-14,000 tons dressed weight. However, there is little known of the growth characteristics of chinook in cage culture. What records do exist are not readily applied to growth modelling due to confounding factors which include unknown stock pedigrees, unknown diet histories, differences among husbandry practices such as grading and feeding, and inadequate routine sampling of growth.

A preliminary growth model for chinook salmon in cage culture was developed by Kreiberg et al. (1988). That study was an improvement over previous knowledge in that diet, cage size, and maximum loading density were standardized, and the groups of fish raised at five different study sites were genetically identical batches representing six B.C. river strains of chinook. Using a general purpose non-linear growth model (Schnute 1981), Kreiberg et al. (1988) showed that within each of the five rearing sites used, growth could be modelled successfully relative to thermal history expressed in Accumulated Thermal Units (ATU) or degree-days, derived by the common hatchery practice of summing once-daily water temperature observations over a period.

However, the model was not as broadly applicable as desired. For a given ATU value, predicted weight was substantially different over the five sites used. Clearly, not all sites recorded the same ATU totals over the grow-out period (approximately 700 d). A northerly site which recorded a lower ATU sum than any other site also logged less growth (but growth followed the same general curve as for other sites, simply not advancing as far). In addition, however, there appeared to be an effect of 'quality' of ATU. A case was noted where one site recorded a relatively high ATU sum yet attained markedly less growth than other sites with slightly lower ATU sums for the same grow-out period.

An examination of temperature data sets suggested that summer water temperatures at some sites may have been high enough to restrain the potential for growth better realized at other sites where identical fish on the same diet experienced more optimal temperatures. It was hypothesized that if 'quality' of ATU could be compensated for by analytical procedure, perhaps the Schnute (1981) model could be used with the existing growth and temperature data sets to make accurate predictions independent of site. The result would be a general growth model for chinook in cage culture in B.C. This report describes one approach taken toward compensating for the effects of differences in seasonal high temperatures when modelling growth.

## METHODS

The procedure selected was the use of a chosen set-temperature or Reflecting-Temperature (RT), above which level observed daily temperatures in the data set from a site would first be reduced by the amount they exceeded the RT before being summed into the ATU data set. For example, for a RT of 15°C, an observed temperature of 16.5°C would be summed as 13.5°C (RT - absolute difference of RT and observed). Temperatures below RT were summed unaltered. This transformation responded specifically to each daily observation in excess of RT, and was magnitude sensitive, thus preferable to a flat-lining or cut-off approach.

The Schnute (1981) model was run again for each site using the original growth data, but with a series of ATU data sets transformed using RTs of 12, 13, 14, 15, 16, 17, 18, and 19°C. If influence of excessively high temperatures on ATU was the principal factor accounting for differences in observed growth, a single RT could be found yielding essentially the same predicted weight at all sites.

## RESULTS AND DISCUSSION

The reflecting-temperature transformation alleviated some of the disparity in predicted growth at different sites. Ranges of predicted weights varied with RT. The smallest range between predicted weights at different sites occurred for an RT between 13 and 14°C, which suggests that the effect on quality of ATU due to excessive temperatures is minimized at this level (Fig. 1). However, the fact that none of the RTs used resulted in identical predicted weights at the five sites suggests the presence of other environmental factors affecting growth.

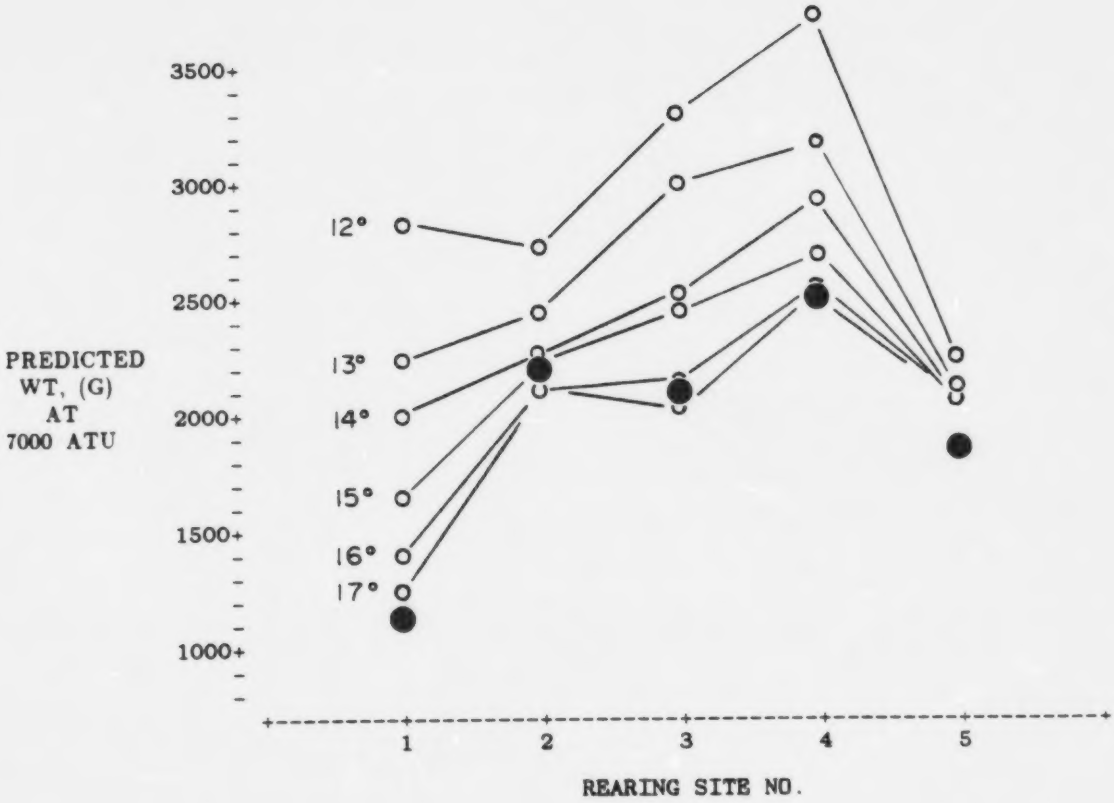


Fig. 1. Mean weight of chinook at 7000 accumulated thermal units (ATU) predicted by Schnute (1981) growth model using data sets transformed using Reflection Temperatures (see text) from 12-17°C for five cage sites in B.C. Closed circles indicate weights predicted from untransformed ATU data (which agree very closely with observed weights).

Temperature (ATU) was initially examined as a basis for predicting growth because Kreiberg et al. (1988) reported that environmental sources contributed approximately 80% of observed variation in mean weights of chinook in cage culture. The present result indicates that compensating for unfavorably high temperatures via the reflecting-temperature approach is insufficient to permit formulation of a general model for chinook growth. Further understanding of other potentially significant components of the rearing environment, for example, feeding techniques, is required.

The reflection-temperature transformation cannot indicate truly the growth which might have been obtained had a particular site experienced other than its actual thermal history. However, in that it effectively doubles the influence of higher temperatures from a modelling standpoint, the RT giving the least difference in predicted weight among different rearing sites may suggest where an upper temperature optimum for growth lies.

Some observations on the principle of reflection-temperature transformations may be made. Since the RT data bases conform to observed temperature data sets except where excesses were recorded, appropriate RT data sets may be useful for predicting growth at a site when seasonal temperature cycles are displaced below normal. That is, site-specific growth models such as reported by Kreiberg et al. (1988) could be used to predict growth despite the temperature profile having been shifted downward from that used to generate the model. Experience has shown that moderately accurate predictions can be made for new rearing sites using Kreiberg et al.'s (1988) model whose temperature profile most closely matches that of the new site.

The approach could also be useful with respect to unfavorably low temperatures occurring sporadically during a grow-out cycle. RT data sets, on the other hand, do not compensate for relative frequency of fluctuation in temperature records like a variance-based approach would. It should be noted that this transformation assumes a symmetrical response to temperature above and below the optimum. As has been shown by Brett et al. (1969) for juvenile salmon, growth falls off sharply above the optimum, hence the RT transformation probably does not adequately adjust for very high temperatures.

## CONCLUSIONS

1) Compensation for differences in thermal history of cage-cultured chinook salmon does not account for all of the observed variation in growth attained among different rearing sites.

2) There is an indication that a temperature of 13-14°C is optimal for growth of chinook salmon in cage culture.

## ACKNOWLEDGMENTS

I am indebted to Dr. C. Clarke who suggested the reflection-point transformation.

## REFERENCES

Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Board Can. 26: 2363-2393.

Kreiberg, H., R. E. Withler and W. C. Clarke. 1988. Early seawater growth of chinook salmon strains at rearing sites in BC. Aquacult. Assoc. Can. Bull. 88-4: 16-18.

Schnute, J. 1981. A versatile growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. 38: 1128-1140.

# EXTENSIVE STARTFEEDING OF MARINE FRY

Kjell E. Naas
Institute of Marine Research
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

## ABSTRACT

Naas, K. E. 1990. Extensive startfeeding of marine fry, p. 137-141. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Startfeeding is one of the most critical phases in the cultivation of marine fishes. Recent progress with startfeeding larvae of cod, turbot, and halibut in extensive systems has induced an increased commercial interest for cultivation of these fishes in Norway. Due to the superior nutritional quality of natural zooplankton, zooplankton-fed larvae grow faster and experience higher survival rates, compared with traditional startfeeding diets, i.e. *Brachionus plicatilis* and *Artemia salina*. Present research focuses on the possibility of managing biological production in the enclosures used for extensive rearing of larvae. The aim is to rear the larvae at high concentrations and to increase the yield of the systems.

## RÉSUMÉ

Naas, K. E. 1990. Extensive startfeeding of marine fry, p. 137-141. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'alimentation initiale compte parmi les étapes les plus critiques de la culture de poissons marins. Les récents progrès réalisés dans l'alimentation initiale des larves de morue, de turbot et le flétan dans des bassins élargis, a provoqué un intérêt accru pour la culture commercialle de ces poissons en Norvège. Étant donné la qualité nutritive supérieure des zooplanctons naturels, les larves alimentées de zooplancton croissent plus vite et ont un taux de survie plus élevé que les larves qui suivent un régime initial traditionnel, c.-à-d. composé de *Brachionus plicatilis* et de *Artemia salina*. Les recherches actuelles visent à déterminer s'il est possible de contrôler la production biologique des enclos utilisés pour l'élevage prolongé de larves. L'objet est d'élever les larves en très fortes concentrations et d'accroître le rendement des bassins.

# INTRODUCTION

## STARTFEEDING - THE BOTTLENECK IN MARINE FISH FRY PRODUCTION

In 1908, Dr. R. Anthony asked, "What is left to be done in the culture of turbot?" He had managed to obtain fertilized eggs from natural spawning, hatched the eggs and reared the larvae on natural zooplankton with 90% survival rates. Almost a century later, the supply of turbot fry is still limited, and fewer than 2,000,000 fry will be produced in 1989.[1]

As for most marine fishes, startfeeding of turbot larvae is the greatest challenge. Most marine fish larvae seem to depend on live prey organisms at the critical stage of first feeding, and the nutritional value of the food is vital for success (Sargent 1976; Watanabe et al. 1983; Dendrinos and Thorpe 1987).

The most common food items for cultured marine fish larvae have been *Brachionus plicatilis* and *Artemia salina*. Both species are easy to cultivate at high concentrations. The problems in marine fish culture using these and other feeds are related to the nutritional value and to the risk of bacterial infection in the startfeeding tanks. For turbot (*Scophtalamus maximus*), cod (*Gadus morhua*), and halibut (*Hippoglossus hippoglossus*), the survival rates using these feeds are generally less than 10% (Turbot: Jones et al. 1981; Person-Le Ruyet et al. 1981; Cod: Gamble and Houde 1984; Howell 1984; Halibut: Mangor-Jensen and Naas, unpubl.).

## EXTENSIVE STARTFEEDING

Extensive startfeeding means that the larave are stocked in a predator-free enclosure where they choose their food from a spectrum of prey organisms. In that way, one leaves the production of prey organisms to nature and the only necessary input to a complete extensive system is nutrients and sunlight. To ensure that sufficient amounts of food are available, the management of an extensive system has to be adjusted to the reproduction rate of the prey organisms. An extensive system can, therefore, be described as an outdoor enclosed water column in which the exchange rate of water is less than the reproduction rate of the prey organisms.

In a true extensive system, all prey organisms are produced within the system, as opposed to an intensive system where the food particles are manufactured and supplied.

Maximum control and security is probably best achieved in an intensive rearing system. In such a context, it is appropriate to consider the extensive system as an early phase in the development of a controlled intensive production system. By means of the extensive method, it is possible to avoid many of the problems connected with startfeeding. The larvae are offered a broad spectrum of potential prey organisms in an almost natural environment and are free to choose the most appropriate prey. In this way, it is possible to learn which organisms the larvae prefer and what nutritional composition of the food is optimal.

## MANAGED BIOLOGICAL PRODUCTION

By using an ecosystem approach in the study of the extensive startfeeding unit, it is possible to increase predictability, control, and yield of the system. In this context, managed biological production is applied to channelize the flow of energy through optimal elements of the food chain with respect to the top predator (fish larva) and thereby increase the yield of the system.

Parameters that will influence the species composition of any trophic level below the top predator can be used to manage the ecosystem towards higher production of fish larvae. The feasibility of the possible parameters to manipulate the system depends on the size of the rearing unit and involves the following: artificial fertilization (amount, composition, and frequency), turbulence, light, temperature, exchance rate, and aeration. The effects of ecosystem manipulations are summarized in Solemdal (1981) and in Grice and Reeve (1982).

# STATUS AND PERSPECTIVES IN REARING MARINE FISH FRY

## COD

The basic elements of extensive production of cod fry were developed in the years 1980-85 (Kvenseth and Øiestad 1984; Øiestad 1985; Øiestad et al. 1987). The idea has been to utilize the highly productive ponds (small landlocked fjords) as feed resources for cod larvae. Potential predators in the enclosed ponds have been killed by rotenone prior to the release of fish larvae and artificial feed has been added when the plankton community has been exhausted, i.e. after metamorphosis of the fish larvae.

---

[1] Information collected at the ICES Working Group meeting; "Mass rearing of juvenile marine fish" at Palavas les Flots, France, June 1989.

In the experimental pond (Hyltropollen, 60,000 $m^3$) at Austevoll, generally between 30 and 50% of the initial cod stock have reached metamorphosis, and between 1 and 2 fry per $m^3$ were harvested in the years 1983-87.

Extensive production of cod fry has been tried commercially since 1987. The promising results from the experimental pond at Austevoll have not been reproduced on a commercial scale. The main problems have been connected with: 1) maintaining a high and stable zooplankton production; 2) estimating the larvae/fry stock; 3) weaning; and 4) harvesting the fry.

During the last 2 yr, a few production experiments have been conducted in submerged plastic enclosures, ranging from 50-600 $m^3$. The food has been natural zooplankton supplied by filtering the surrounding water. This method has been more or less adapted from the turbot fry production method and is, of course, very dependent on the concentration of zooplankton at the production site. Cod fry are very cannibalistic. If the food supply is limited, and there is a high concentration of cod fry in the bags, the result is a high rate of cannibalism (Folkvord 1989).

## TURBOT

Several commercial companies in Norway produced turbot fry semi-intensively in 1988 and will continue to do so in 1989. By this method, natural zooplankton is collected from the surrounding waters and added to the startfeeding tank or bag (Meeren 1987). The results so far have demonstrated that the availability of zooplankton is the most important limiting resource, and that highly productive sites (ponds) determine whether the result is a success or failure.

In the semi-intensive production concept, the pond enclosure is exclusively a food production unit. Our empirical data suggest that the zooplankton biomass is between one and two orders of magnitude higher in the ponds than in the free fjord waters. It is also possible to increase the zooplankton production in these pond enclosures by manipulations (as described earlier) and thereby maintain a high and stable food resource.

The turbot fry reared on a natural zooplankton diet have generally been more viable and less malpigmented than fry reared on an intensive diet of *Brachionus plicatilis* and *Artemia salina* (Quantz et al. 1981; Witt et al. 1984; Paulsen 1988). Most of the turbot fry on the market in 1989 will probably be produced using a semi-intensive production line. Producers have documented survival rates between 10 and 40% using this method.

The problems the turbot fry producers are facing are similar to those of the cod fry producers, i.e. 1) a stable and high zooplankton supply, 2) the quality of the artificial weaning diet, and 3) diseases.

## HALIBUT

As for cod and turbot, the most promising results with startfeeding halibut larvae have been obtained by using natural zooplankton (Naas et al. 1987). The first two halibut fry were artificially reared in 1985 in submerged plastic enclosures supplied with filtered zooplankton. After the development of the more elaborate silos for incubating the larvae through the period of yolk sac absorption (1987-88) (Rabben et al. 1987), the increased numbers of functional larvae have made startfeeding experiments possible. So far, the results suggest that the halibut larva is an opportunist and capable of eating a diverse diet, and that low survival rates are connected with the nutritional quality of the food offered rather than the feeding rate.

In 1989, many production experiments were conducted in smaller mesocosms, which have been prepared with optimum zooplankton composition prior to release of fish larvae. These mesocosms (tanks or bags ranging from 5-300 $m^3$) have been inoculated with phytoplankton and zooplankton and added artificial fertilizers. After the larvae have grazed down the zooplankton population, prey organisms have been added, either filtered zooplankton from the sea or non-enriched *Artemia salina* nauplii. The hypothesis is that the larvae must have a very high quality diet, rich in highly unsaturated fatty acids and free amino acids, for a limited period of time, 5-10 d. In a functional ecosystem, like the mesocosm, the prey organisms will not lose their nutritional quality during the period before they are eaten.

As the fish larvae develop, they are probably more capable of utilizing food particles of low quality. It has been reported (K. Hjelmeland, FORUT, Tromsø, Norway, pers. commun.) that, for example, the trypsin level in the gut of halibut larvae is low compared to that in other fish larvae. This indicates a need for free amino acids in the initial diet, and that the ability to utilize macromolecules increases with the development of the enzymes in the gut.

In 1988, fewer than 2000 halibut larvae were reared successfully beyond metamorphosis. In 1989, 10,000 fry were produced, of which approximately 80% were from commercial production experiments.

## REFERENCES

Anthony, R. 1908. The cultivation of turbot. Proceedings of the Fourth International Fishery Congress, Washington, US, 1908.

Dendrinos, P., and J. P. Thorpe. 1987. Experiments on the artificial regulation of the amino acid and fatty acid content of food organisms to meet the assessed nutritional requirements of larval, postlarval and juvenile Dover sole (*Solea solea* L.). Aquaculture 61: 121-154.

Folkvord, A. 1989. Growth and cannibalism of cod fry (*Gadus morhua* L.) in intensive systems, p. 133-138. In N. DePauw, E. Jaspers, H. Ackefors, and N. Wilkins (ed.) Aquaculture - a biotechnology in progress. European Aquaculture Society, Bredene, Belgium.

Gamble, J. C., and E. D. Houde. 1984. Growth, mortality and feeding of cod (*Gadus morhua* L.) larvae in enclosed water columns and in laboratory tanks. Flødevigen Rapportser 1: 123-143.

Grice, G. D., and M. R. Reeve. 1982. Marine Mesocosms. Springer Verlag, New York, 430 p.

Howell, B. R. 1984. The intensive rearing of juvenile cod (*Gadus morhua* L.). Flødevigen Rapportser 1: 657-675.

Jones, A., R. A. Prickett, and M. T. Douglas. 1981. Recent developments in techniques for rearing marine flatfish larvae, particularly turbot (*Scophtalamus maximus* L.) on a pilot commercial scale. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 178: 522-526.

Kvenseth, P. G., and V. Øiestad. 1984. Large-scale rearing of cod fry on the natural food production in an enclosed pond. Flødevigen Rapportser 1: 645-655.

Meeren, T. v.d. 1987. Studier av næringsinntak, vekst og overlevelse hos larver av piggvar (*Scophtalamus maximus* L.) under intensivt oppdrett. M.Sc. Thesis, Univ. of Bergen, Norway, 105 p.

Naas, K. E., L. Berg, J. Klungsøyr, and K. Pittman. 1987. Natural and cultivated zooplankton as food for halibut (*Hippoglossus hippoglossus*) larvae. Int. Counc. Explor. Sea C.M. 1987/F:17.

Paulsen, H. 1988. Current status of turbot culture. Proceedings of the third international symposium on the reprpoductive physiology of fish. St. John's, Newfoundland, Canada, 2-7 August 1987.

Person-le Ruyet, J., D. L'Elchat, and G. Nedelec. 1981. Research on rearing turbot (*Scophtalamus maximus* L.): results and perspectives. J. World Maricult. Soc. 12: 143-152.

Quantz, G., D. Kuhlmann, U. Witt, W. Nellen, and J. Lenz. 1980. Rearing of turbot larvae (*Scophtalamus maximus* L.) on cultured food organisms: results from the 1979 and 1980 rearing seasons. Int. Counc. Explor. Sea C.M. 1987/F:32.

Rabben, H., A. Jelmert, and I. Huse. 1987. Production experiment of halibut fry (*Hippoglossus hippoglossus* L.) in silos. Int. Counc. Explor. Sea C.M. 1987/F:42.

Sargent, J. R. 1976. The structure, metabolism and function of lipids in marine organisms, p. 149-212. In D. C. Malins and J. R. Sargent (ed.) Biochemistry and biophysical perspectives in marine biology. Vol. III, Academic Press, London.

Solemdal, P. 1981. Enclosure studies - overview. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 178: 117-120.

Watanabe, T., C. Kitajima, and S. Fujita. 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34: 115-134.

Witt, U., G. Quantz, D. Kuhlmann, and G. Kattner. 1984. Survival and growth of turbot larvae (*Scophtalamus maximus* L.) reared on different food organisms with special regard to long-chained polyunsaturated fatty acids. Aquacult. Engineer. 3: 177-190.

Øiestad, V. 1985. Predation on fish larvae as a regulatory force, illustrated in mesocosm studies with large groups of larvae. Northwest Atl. Fish. Organ. Sci. Counc. Stud. 8: 25-32.

Øiestad, V., T. Pedersen, A. Folkvord, Å. Bjordal, and P. G. Kvenseth. 1987. Automatic feeding and harvesting of juvenile Atlantic cod (*Gadus morhua* L.) in a pond. Model. Identif. Control 8: 39-46.

# COD ENHANCEMENT EXPERIMENTS IN NORWAY

Terje Svåsand
Directorate of Fisheries
Institute of Marine Research
Division of Aquaculture
P. O. Box 1870, N-5024 Nordnes-Bergen, Norway

## ABSTRACT

Svåsand, T. 1990. Cod enhancement experiments in Norway, p. 143-151. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The prospects of enhancement of coastal cod populations were studied at four localities in Norway. The objectives of the projects were to compare reared and wild cod and to investigate if the release of reared cod can increase the yield from coastal cod populations. The enhancement experiments started after a breakthrough in mass rearing of cod fry in 1983. Different tags and marks such as Floy anchor tags, oxytetracycline, freeze branding, and internal steel tags have been used to mark the reared cod before release. The present results reveal only minor differences between wild and reared cod. However, loss of rare alleles in the broodstock compared with the natural cod population and differences in behavior of newly released cod have been reported. The transition from the rearing environment to the release area is probably critical and should be investigated further. At one of the release areas, reared cod contributed between 20 and 60% of the 1983-86 year-classes at the time of release. The reared cod moved little after release and no indications of spawning migration have been found. The natural mortality after release of reared cod was high and the fishing mortality low before recruiting to the local fishery as II-group. Older cod (II+ groups) had a reverse mortality pattern (low natural mortality and high fishing mortality). The mortality in the pre-recruit period is suggested to be both size and density dependent. The first approach to model the potential production of cod has been reported, and the continuations of these investigations will probably give an answer to the question whether artificial propagation can increase the yield of cod in coastal areas.

## RÉSUMÉ

Svåsand, T. 1990. Cod enhancement experiments in Norway, p. 143-151. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Les perspectives de mise en valeur des populations côtières de morue ont été étudiées à quatre endroits de la Norvège. Ces études avaient pour objet de comparer la morue d'élevage et la morue sauvage et d'étudier si l'ensemencement de morue d'élevage peut augmenter le rendement des populations côtières de morue. Les expériences de mise en valeur ont commencé après que l'on eut réalisé une grande découverte dans l'élevage des alevins de morue en 1983. Le marquage de la morue avant sa remise à l'eau se fait de différentes façons, notamment par les marques Floy, par l'oxytétracycline, par le cryomarquage et par des étiquettes internes en acier. Les résultats obtenus jusqu'à présent ne révèlent que de faibles différences entre la morue sauvage et la morue d'élevage. Cependant, la perte d'allèles rares dans le stock d'élevage se compare à celle des populations indigènes et on a signalé des écarts de comportement chez la morue qui venait d'être libérée. La transition entre le milieu d'élevage et la zone de remise à l'eau est sûrement une étape critique au développement et devrait être étudiée plus à fond. À l'un des lieux de remise à l'eau, la morue cultivée a contribué de 20 à 60 pour 100 des classes d'âge de 1983 à 1986. La morue cultivée s'est très peu déplacée après sa libération et on n'a trouvé aucune preuve de migration aux

frayères. Avant de devenir le recrutement de la pêche locale, la morue libérée, âgée de deux ans, a subi un taux de mortalité naturelle élevée et un faible taux de mortalité due à la pêche. Les morues plus âgées (plus de deux ans), ont connu le contraire: faible mortalité naturelle et forte mortalité due à la pêche. On pense que la mortalité subie pendant la période précédant le recrutement pourrait dépendre de la taille et de la densité du poisson. On a fait état d'une première approche visant à modeler la productivité possible de la morue, et la poursuite de ces études nous permettra probablement de déterminer si la propagation artificielle peut augmenter le rendement de la morue dans les zones côtières.

## INTRODUCTION

In 1983, Norwegian scientists succeeded in mass rearing of juvenile cod in a natural seawater pond in western Norway (Øiestad et al. 1985). This breakthrough opened up new possibilities for enhancing natural cod populations. The first release experiment was started at Austevoll in western Norway in 1983 (Svåsand et al. 1990), and in 1985, this research was scaled up when the Norwegian Council of Fisheries Research decided to initiate a nation-wide enhancement program with projects at three different regions in Norway (Fig. 1). Institutions such as the Institute of Marine Research, the University of Bergen, and the University of Tromsø became involved and studies in marine ecology, fishery biology, and hydrography were started. In this paper, I summarize the results to date from the ongoing enhancement experiments on coastal cod in Norway. However, the three projects started in 1985 are still in progress and only few results have been published.

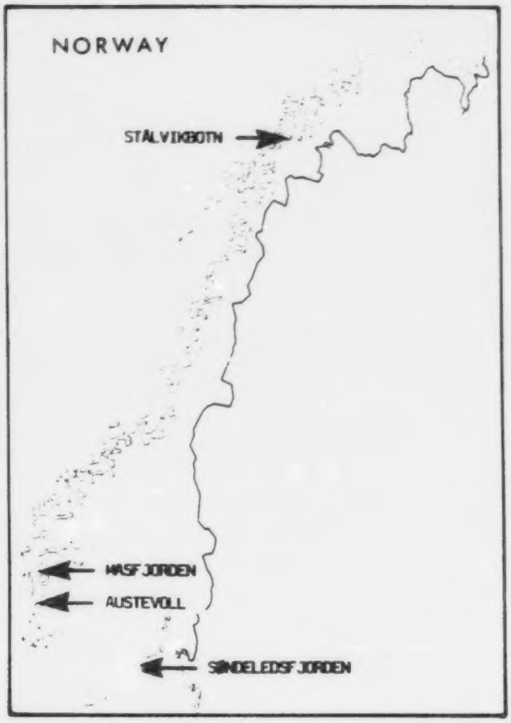


**Fig. 1. The locations of areas where the prospects of cod stock enhancement are being studied in Norway. Reared cod were released at Austevoll in an open coastal area and in a land-locked fjord, while the other projects are all conducted in fjord areas.**

## TAGGING

When studying migration and when information from fishermen are needed, Floy anchor tags have been used to mark reared fish before release. These tags can be used on cod larger than about 15 cm with low tagging mortality, but shedding of tags has been a problem (unpubl. data). The cost of the tag (3 NOK or $0.55 Can.) and the laborious tagging process have limited the use of Floy anchor tags to relatively small releases (<20,000). Experiments with freeze branding of juvenile cod have shown that the mark disappears after some months, and the method is therefore not recommended for future use on cod (Jarle T. Nordeide, Institute of Marine Research, pers. commun.). Cod larger than about 10 cm can be tagged with internal steel tags placed in the body cavity (Moksness and Øiestad 1984; Svåsand et al. 1987) and both tagging mortality and loss of tags have been acceptably low (unpubl. data). However, since the use of a tag indicator or visual inspection of the body cavity is necessary to detect the tag, and when considering that the tagging time is at least as long as for Floy anchor tags, the latter method is preferable.

In 1988, nearly 90,000 0-group cod marked with oxytetracycline (OTC) were released in Masfjorden (Jarle T. Nordeide, pers. commun.). OTC binds with proteins in the blood and is incorporated in newly formed and mineralizing bone and cartilage (Frost et al. 1961) and the marks can be visualized using ultraviolet light (Kobayashi et al. 1964). The centrum of cod is used for detection of OTC-marked individuals. Several studies have reported use of OTC to mark fish; however, none on cod. Future investigations concerning selection of dosages of OTC and verification of long-time duration of the mark on cod should therefore be carried out before this method can be recommended on this species.

Recently, a genetic marker for cod was developed (Jørstad et al. 1987). This mark which is detectable at all life stages might be of great importance in both early life stage investigations and in future large-scale release programs with cod (Svåsand et al. 1989).

## COMPARATIVE STUDIES BETWEEN WILD AND REARED COD

There are many published examples of differences between reared and wild individuals and examples of morphological, behavioral, physiological and biochemical differences have been reviewed by Blaxter (1976). Several references to more recent studies showing differences in feeding behavior, survival, and genetic characters between wild and reared fish are given in Nordeide and Salvanes (1989) and Svåsand et al. (1990). The present results from studies comparing wild and reared cod are summarized below.

### GENETICS

Genetic investigations were incorporated in the cod enhancement experiment at Austevoll, and studies of allele frequencies in four loci (Hbl, LDH-3, PGM and PGI-1) were conducted. Two rare alleles, PGM(150) and PGM(70), found in the wild population turned out to be lacking in the broodstock consisting of 74 mature cod caught in the release area. No other genetic differences were detected, neither between broodstock and offspring, nor during a 3-yr period after release (Svåsand et al. 1990). However, the loss of the two rare alleles indicates that the number of cod in the broodstock was too low and a higher number of spawners should therefore be used to produce juveniles for future release experiments.

### GROWTH

Several authors have reported similar growth of reared and wild cod of equal age in the same area (Moksness and Øiestad 1984; Svåsand and Kristiansen 1985; Kristiansen 1987; Svåsand et al. 1987; Kristiansen and Svåsand 1990 (results given in Fig. 2); Svåsand et al. 1990). No significant differences in growth rates between different year-classes of II+ cod were found at one of the release areas at Austevoll (unpubl data). However, differences in growth of reared cod released at

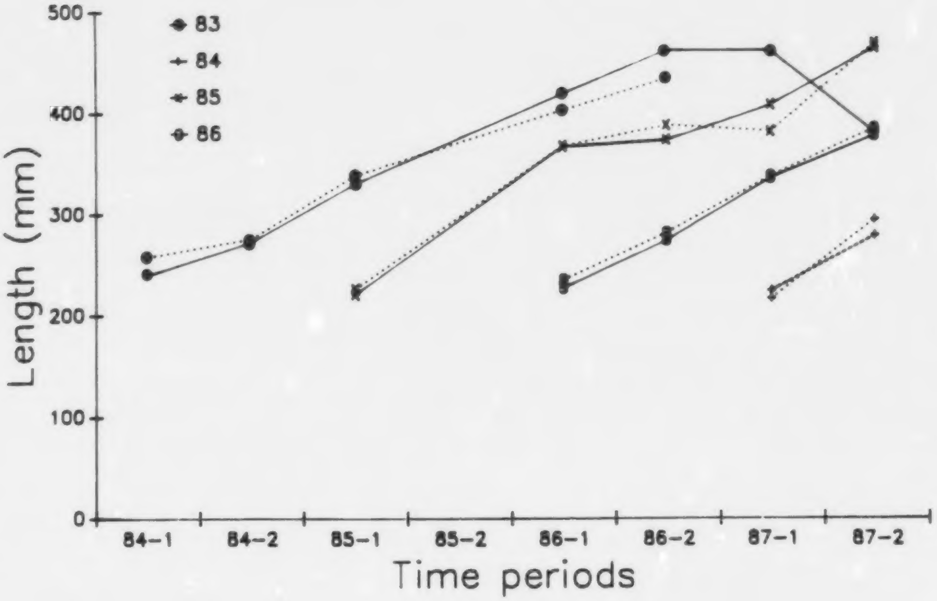


Fig. 2. Mean length in different sampling periods (84-1: January-June 1984; 84-2: July-December 1984;...; 87-2: July-December 1987) of reared (dotted line) and wild (solid line) cod of the 1983-86 year-classes in Heimarkspollen at Austevoll (from Kristiansen and Svåsand 1990). Significant differences in mean length between reared and wild cod were found only for the 1985 year-class in sampling period 86-1 (Student t-test, P<0.05). It should be noted that number of reared (0-98) and wild fish (1-166) in the different sampling period were different. Only one fish was sampled from the 1983 year-class in sampling period 87-1. The standard deviation of the length in the sampling periods varied between 25-107 mm for reared and 25-102 mm for wild cod (n > 1).

different areas have been found, and the growth rates of cod in a closed area (landlocked fjord) were lower than the corresponding growth rates of cod released in more open coastal areas (Kristiansen 1987). This indicates that the growth of cod varies between areas, but that the growth rates of wild and reared cod within areas are similar.

## FEEDING AND BEHAVIOR

Similar feeding preferences (Svåsand and Kristiansen 1985; Kristiansen 1987) and weight of the stomach contents between wild and reared cod (Kristiansen 1987) have been reported, based on stomach content analyses of I+ cod. However, Nordeide and Salvanes (1989) found differences in feeding behavior between wild and newly released reared 0-group cod. This indicates that reared fish need some time after release to adapt to the new environment.

Nordeide and Salvanes (1989) found that densely stocked 0-group cod were heavily preyed upon just after release, and Nordeide and Svåsand (1990) reported differences in behavior of juvenile reared and wild cod towards a potential predator. The first period after transition from the production unit to the release area might, therefore, be critical. Development of methods giving quicker adaptation to and dispersal within the release environment, and thereby increased survival, should be investigated in future enhancement programs.

## MIGRATION OF REARED COD

Several studies have shown that the majority of reared cod released as 0-group in sheltered coastal and fjord areas remained in the locality of release (Moksness and Øiestad 1984; Nordeide and Salvanes 1988; Svåsand and Kristiansen 1990a). Svåsand and Kristiansen (1990a) found that 96% of the recaptures from a release experiment at Austevoll in western Norway in 1983 were captured in shallow nearshore waters less than 10 km from the release sites. In this experiment, 19,000 0-group cod were tagged, and up to 1 January 1988, more than 3000 tags had been returned, mainly from fishermen and leisure anglers. Updated results from this experiment are shown in Fig. 3. A weak size-dependent dispersion (increased dispersion with increased fish size) was indicated (Fig. 3a), but the movement showed no marked preferential directionality (Svåsand and Kristiansen 1990a). The majority of the cod in the Austevoll region reach maturity between 3 and 4 yr of age (Svåsand and Kristiansen 1985; Kristiansen 1987; Svåsand et al. 1990), and the results in Fig. 3b show that mature cod also remained in the area of release. Similar results have been reported for wild coastal cod at the Norwegian Skagerak Coast (Løversen 1946), and for wild coastal cod tagged as juveniles in sheltered coastal and fjord areas in western Norway (Nordeide and Salvanes 1988; Godø et al. 1986; Svåsand 1990).

## MORTALITY OF REARED COD AFTER RELEASE

Svåsand and Kristiansen (1990b) found high natural and low fishing mortality rates of young cod (0- and I-groups), and low natural and high fishing mortality rates in older cod (II+ groups). The estimated instantaneous coefficient of total mortality (Z) from release as 0- and I-groups to the II-group stage (pre-recruit mortality) varied from 1.0 to 1.6 $yr^{-1}$. The high mortality in this period was attributed to predation and cannibalism (natural mortality), and the results suggest both size and density-dependent effects (Svåsand and Kristiansen 1990b). Survival in the pre-recruit period increased with increased size at release and decreased with increased total 0-group abundance of wild and reared cod. The instantaneous coefficient of natural mortality (M) of cod larger than 30 cm (II+ cod) was estimated as 0.22 $yr^{-1}$ (ca. 20% $yr^{-1}$). After recruitment to the local fishery as II-group, the mean instantaneous coefficient of fishing mortality (F) was estimated as about 0.6 $yr^{-1}$ (ca. 45% $yr^{-1}$). The mortality patterns of wild coastal cod have not been investigated, but similar mortality rates have been reported for the North Sea cod (Anon. 1988).

## EFFECTS OF RELEASES OF REARED COD AND CARRYING CAPACITY

From 1983-86, nearly 50,000 reared cod were tagged (primarily with Floy anchor tags) and released in the Austevoll area, and up to 1 June 1989, 8000 (16%) cod had been reported recaptured (unpubl. data).

Year-class abundances of total 0-group cod (wild and reared) immediately after the reared groups of cod had been released (September-

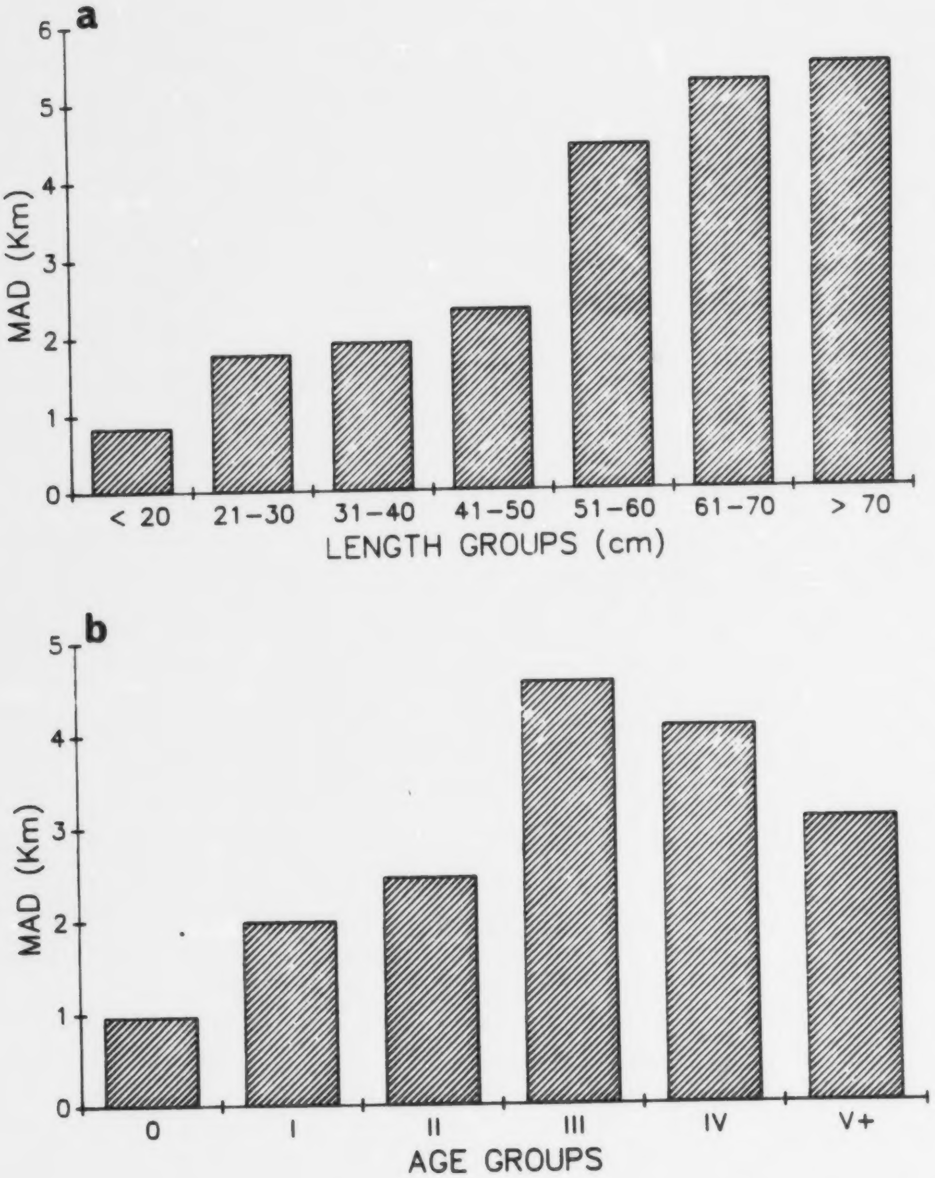


**Fig. 3.** Mean absolute displacement distances (MAD) of various length (a) and age groups (b) of recaptured cod from a release experiment at Austevoll in western Norway in 1983 (drawn from data in Svåsand and Kristiansen (1990a) and updated recapture data). Only data from fishermen and leisure anglers are included (see text for more information). MAD: mean displacement distances (straight line from release to recapture) of the fish in a group.

December) were estimated in Heimarkspollen, a small, landlocked fjord (2.9 km$^2$) at Austevoll (Kristiansen and Svåsand 1990) by the "Petersen method" (Seber 1982, Chapter 3.1), with the modified estimator of the number of the population given by Chapman (1951). The results showed that reared cod contributed significantly (20-60%) to the 1983-86 year-classes (Kristiansen and Svåsand 1990). These results show that it is possible to increase the local abundance of 0-group cod by artificial propagation. However, if the survival of 0-group cod is density dependent as suggested by Svåsand and Kristiansen (1990b), the releases of reared cod might decrease the pre-recruit survival of both wild and reared cod and thereby also the potential yield per recruit. The wild 0-group abundance should, therefore, be measured before deciding on the number to be released, and the number released should be adjusted to the carrying capacity of the release area. The carrying capacity for cod is now being investigated in the Masfjord project (Salvanes 1986), and several contributions to this study were recently presented at an international symposium on extensive aquaculture in France (Fosså 1989; Giske et al. 1989; Kaartvedt 1989; Nordeide and Salvanes 1989). If this ecosystem study is followed up, the question as to whether propagation can increase the yield of cod in coastal areas might be answered within the near future.

## FUTURE PROSPECTS

If the conclusion of the cod enhancement projects is that it is possible to increase the output from the inshore cod fisheries by release of reared cod, some important questions still remain to be answered before this kind of extensive aquaculture can be a reality. For example, the output from an enhancement program will depend on the management routines (Ulltang 1984), and Svåsand and Kristiansen (1990b) showed that the fishing mortality was high from the II group stage (length > ca. 30 cm). Before starting larger enhancement programs, it will therefore be important to investigate how yield per recruit depends on the fishing pattern (number and types of gear).

A full-scale enhancement program will also demand a continuous supply of low cost cod fry. Today, this seems to be a problem, but a great effort has been put into optimizing the rearing techniques (Blom et al. 1989). Finally, there will also be the question whether a cod enhancement program will be economically feasible (Sandberg and Flåm 1988).

In conclusion, the results from the cod enhancement projects have increased our general knowledge about coastal cod populations and the biological basis for future cod enhancement. However, there are still some questions that have to be answered before cod enhancement can be a reality in Norwegian coastal and fjord areas.

## ACKNOWLEDGMENTS

I thank Arild Folkvord, Marianne Holm, Tore S. Kristiansen and Jarle T. Nordeide for valuable and critical comments on earlier versions of the manuscript.

## REFERENCES

Anon. 1988. Report of the North Sea roundfish working group. Int. Counc. Explor. Sea C.M. 1988/Assess:21, 248 p.

Blaxter, J. H. S. 1976. Reared and wild fish - how do they compare?, p. 11-26. In G. Persone and E. Jaspers (ed.) 10th European Symposium on Marine Biology, Vol. 1, Universal Press, Wettern, Belgium.

Blom, G., T. S. Kristiansen, H. Otterå, and T. Svåsand. 1989. Relationship between production of zooplankton and productin of cod fry (*Gadus morhua* L.) - modelled from studies in a marine semi-enclosed ecosystem. Int. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 1: 18 p.

Chapman, D. G. 1951. Some properties of the hypergeometric distribution with applications to zoological censuses. Univ. Calif. Public Stat. 1: 131-160.

Fosså, J. H. 1989. Cod enhancement experiment in Masfjorden: production of, and gadoid predation on, *Gobiusculus flavescens* (Fabricius). Int. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 14: 14 p.

Frost, H. M., A. R. Villanueva, H. Roth, and S. Stanisavljevic. 1961. Tetracycline bone labelling. J. New Drugs 1: 206-216.

Giske, J., D. L. Aksnes, U. Lie, and S. Wakili. 1989. Cod enhancement experiment in Masfjorden: computer simulation of pelagic production and its consequences for production of released 0-group cod. Int. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 16: 14 p.

Godø, O. R., T. I. Halland, and P. Ågotnes. 1986. Tagging experiment results on cod in western Norway fjord areas. Int. Counc. Explor. Sea C.M. 1986/G:80, 7 p.

Jørstad, K. E., V. Øiestad, O. I. Paulsen, K. Naas, and Ø. Skaala. 1987. A genetic marker for artificially reared cod (*Gadus morhua* L.). Int. Counc. Explor. Sea C.M. 1987/F:22, 10 p.

Kobayashi, S., R. Yuki, T. Furui, and T. Kosugiyama. 1964. Calcification in fish and shellfish. I. Tetracycline labelling patterns on scale, centrum and otolith in young goldfish. Bull. Jpn. Soc. Sci. Fish. 30: 6-13.

Kristiansen, T. S. 1987. Vekst og ernæring til utsatt, oppdrettet torskeyngel og vill torsk (*Gadus morhua* L.) i Heimarkspollen, Austevoll. Cand. Scient. thesis, Dept. Fish. Biol., Univ. Bergen, Norway, 134 p.

Kristiansen, T. S., and T. Svåsand. 1990. Enhancement studies on coastal cod in western Norway. III. Inter-relationships between reared and indigenous cod in a nearly landlocked fjord. J. Cons. int. Explor. Mer (in press).

Kaartvedt, S. 1989. Cod enhancement experiment in Masfjorden: macroplankton and micronekton as a potential food resource in fjord systems. Int. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 15: 12 p.

Løversen, R. 1946. Torskens vekst og vandring på Sørlandet. FiskDir.Skr. Ser.HavUnders 8: 1-27.

Moksness, E., and V. Øiestad. 1984. Tagging and release experiment on 0-group coastal cod (*Gadus morhua* L.) reared in an outdoor basin, p. 787-794. In E. Dahl, D. S. Danielssen, E. Moksness and P. Solemdal (ed.) The propagation of cod, *Gadus morhua* L. Flødevigen rapportser, 1984, 1.

Nordeide, J. T., and A. G. V. Salvanes. 1988. The migration of coastal cod (*Gadus morhua* L.) tagged in a fjord of western Norway. In. Counc. Explor. Sea C.M. 1988/G:5, 14 p.

Nordeide, J., and A. G. V. Salvanes. 1989. Cod enhancement experiment in Masfjorden: migration and feeding of densely stocked cod (*Gadus morhua* L.) and stomach content and abundance of potential predators. Int. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 13: 13 p.

Nordeide, J., and T. Svåsand. 1990. The behaviour of wild and reared juvenile cod, *Gadus morhua* L., towards a potential predator. Aquacult. Fish. Manag. (in press).

Salvanes, A. G. V. 1986. Preliminary report from a study of species composition, size composition and distribution of the fish in a fjord of western Norway based on regularly conducted experimental fishing and catch statistics during one year. Int. Counc. Explor. Sea C.M. 1986/G:70, 10 p.

Sandberg, P., and S. D. Flåm. 1988. The rent of coastal water ranching. Discussion Pap. Ser. 6/1988, Institute of Fisheries Economics, Norwegian School of Economics and Business Administration, N-5035 Bergen, Norway.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters (2nd edition). Charles Griffin and Co. Ltd., London and High Wycombe, 654 p.

Svåsand, T. 1990. Comparisons of migration patterns of wild and recaptured reared coastal cod (*Gadus morhua* L.) released in a small fjord in western Norway. Aquacult. Fish. Manag. (in press).

Svåsand, T., K. E. Jørstad, and T. S. Kristiansen. 1990. Enhancement studies on coastal cod in western Norway. I. Recruitment of wild and reared cod to a local spawning stock. J. Cons. int. Explor. Mer (in press).

Svåsand, T., K. E. Jørstad, T. S. Kristiansen, and G. Blom. 1989. Application of genetic markers for early life history investigations on cod (*Gadus morhua* L.). Inc. Counc. Explor. Sea, Symposium on the ecology and management aspects of extensive mariculture 76: 8 p.

Svåsand, T., and T. S. Kristiansen. 1985. Release of artificially reared 0-group coastal cod (*Gadus morhua* L.) in a landlocked fjord in western Norway. Int. Counc. Explor. Sea C.M. 1985/F:10, 12 p.

Svåsand, T., and T. S. Kristiansen. 1990a. Enhancement studies on coastal cod in western Norway. II. Migration of reared coastal cod. J. Cons. int. Explor. Mer (in press).

Svåsand, T., and T. S. Kristiansen. 1990b. Enhancement studies on coastal cod in western Norway. IV. Mortality of reared cod after release. J. Cons. int. Explor. Mer (in press).

Svåsand, T., T. S. Kristiansen, and H. Næss. 1987. Tagging experiments on artificially reared 0-group coastal cod (*Gadus morhua* L.) in western Norway - results from the releases in 1984. Int. Counc. Explor. Sea C.M. 1987/F:25, 15 p.

Ulltang, Ø. 1984. The management of cod stocks with special reference to growth and recruitment overfishing and the question whether artificial propagation can help to solve management problems, p. 795-817. In E. Dahl, D. S. Danielssen, E. Moksness and P. Solemdal (ed.) The propagation of cod, *Gadus morhua* L. Flødevigen rapportser, 1984, 1.

Øiestad, V., P. G. Kvenseth, and A. Folkvord. 1985. Mass production of Atlantic cod juveniles (*Gadus morhua* L.) in a Norwegian salt water pond. Trans. Am. Fish. Soc. 14: 590-595.

# INTENSIVE PRODUCTION OF HALIBUT FRY

A. Mangor-Jensen, T. Harboe, S. Tuene,
K. Boxaspen and L. Skjolddal
Institute of Marine Research
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

## ABSTRACT

Mangor-Jensen, A., T. Harboe, S. Tuene, K. Boxaspen, and L. Skjolddal. 1990. Intensive production of halibut fry, p. 153-159. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

A production line for full-scale intensive production of halibut fry (*Hippoglossus hippoglossus*) is described. The experiments demonstrate that halibut larvae can be reared at low temperature through the yolk-sac stage in small closed systems. However, initial feeding of halibut larvae on the cultivated zooplankton species *Artemia* and *Brachionus* in closed systems has not given satisfactory results in spite of application of standardized zooplankton enrichment with marine oils (HUFA's).

## RÉSUMÉ

Mangor-Jensen, A., T. Harboe, S. Tuene, K. Boxaspen, and L. Skjolddal. 1990. Intensive production of halibut fry, p. 153-159. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Le document ci-dessus décrit une chaîne de production employée dans l'élevage intensif à grande échelle des alevins de flétan (*Hippoglossus hippoglossus*). Les expériences démontrent que les larves de flétan peuvent être élevées à basse température, durant l'étape embryonnaire, dans de petits bassins fermés. Cependant, le régime initial des larves de flétan, composé des espèces de zooplancton cultivées *Artemia* et *Brachionus*, n'a pas donné de résultats satisfaisants malgré l'application d'huile d'animaux marins, une source d'engrais pour le zooplancton.

## INTRODUCTION

Artificial production of marine fish fry involves intensive culture in small rearing systems which have a high degree of controllability and in which the biological components are reproducible.

The overall aim for an intensive production program is to develop a system that is reproducible, is independent of environmental variables, is independent of seasonal variations in natural occurring prey organisms and, most importantly, is able to produce a predictable number of fry.

In the intensive production of fish fry, it is implicit that the feed organisms are cultivated. Turbot fry production in central Europe has been based on such methods but, unfortunately, the actual prey organisms in question have proved to be of poor nutritional quality. Much effort is being placed on research in this field on a world-wide basis.

Production of fry has been, and most certainly will continue to be, the greatest problem in the domestication of halibut. There are, however, several problems that are hidden behind the term "fry production." Production of fry includes broodstock management, egg and sperm production, incubation technology of eggs and yolk-sac larvae, and feeding beyond metamorphosis.

Experiments of rearing of halibut larvae in intensive culture systems were first conducted in the early 1970s (Solemdal et al. 1974). The rearing units have been modified and rebuilt to improve the systems. The concept of closed systems with temperature control has been most used over the last 2 yr, at least during the yolk-sac period. In contrast with the ancient system of usbmerged plastic bags in seawater (Rabben et al. 1986), the systems now in use are more often land-based indoor systems.

The presence of biological and technical problems in first feeding of marine fish larvae are obvious from the low production volume. The knowledge that has been obtained through farming of salmonids does not seem to be applicable for halibut.

In this investigation, a full-scale setup for halibut fry production has been designed and constructed. A major part of the work was based on observation of the larvae under varying environmental conditions. Morphological, physiological, and bacteriological experiments were also conducted.

So far, the production volume has been modest, although the results from the 1989 season have been promising. Compared with other marine species, the halibut has some peculiarities and environmental demands during early rearing stages that have caused problems (Pittman et al. 1987).

In nature, the halibut spawns at great depths during the period January-April (Haug et al. 1984). The eggs are large and transparent and float freely in the water column (Rollefsen 1934). In contrast with their flatfish relatives, Atlantic halibut eggs do not ascend to the surface, but remain at depths greater than 10 m (Haug et al. 1984). The reasons for this have been much discussed, but we now know that these eggs are able to regulate their buoyancy in response to light (Valkner and Mangor-Jensen, unpubl.). When subjected to light, the eggs become heavier and tend to sink unti lthe light is turned off.

After a period corresponding to 90-100 day degrees, a 10-mm transparent larva hatches from the egg (Blaxter et al. 1983). At the time of hatching, the larva is comparatively and suprisingly poorly developed, and enters a long-lasting yolk-sac stage exhibiting almost no activity. The time for complete yolk-sac absorption varies between 180 and 220 day degrees at 5-7°C (Pittman et al. 1987). During this period, the larvae are fragile and large mortalities are occasionally observed due to stress factors such as bacteria, turbulence, temperature changes, and light. The problem of first feeding of halibut larvae in intensive culture systems has not been solved. The number of fry produced in such systems in 1988 was less than 250. These fry also suffered from malpigmentation and poor growth. There are two prevailing theories to explain these difficulties: 1) the live prey organisms contain insufficient amounts of essential nutritional compounds such as highly unsaturated fatty acids (HUFA's) (Leger et al. 1986; Watanabe et al. 1983); 2) the predator-prey interactions in such systems are suppressed due to environmental factors such as light regimes and container volume (Griffiths 1975).

The research on intensive rearing of halibut has, however, given results that are most valuable for extensive production.

## MATERIALS AND METHODS

Eggs and milt were stripped from ripe specimens of the Atlantic halibut (*Hippoglossus hippoglossus* L.) according to the procedure described by Rabben (1987).

The eggs and milt were obtained by applying gentle manual pressure to the fish over the abdominal cavity. Immediately after stripping, the eggs were fertilized by adding a milt-seawater mixture.

The eggs were incubated in tanks supplied with an upwelling water system to provide dispersion (Fig. 1). The egg incubators received filtered seawater (0.2 µm) from 60 m depth. The water was not thermally adjusted since temperature at this depth is relatively constant during the spawning season (Fig. 2). The egg incubators were tended every day in order to remove and count dead eggs. For this purpose, the flow was turned off and 20 L of high salinity water (40 ppt) was introduced through the bottom inlet. This bottom layer of dense water separated dead eggs and debris from the live material since live eggs float and dead eggs sink. After about 15 min, dead eggs were drained out, and the flow turned on. Shortly prior to hatching (day 10 after fertilization), the eggs were collected from the hatching tank and transferred to larval rearing units. The collection of eggs was accomplished by increasing the salinity in the tank until 100% egg buoyancy occurred. The eggs were then sampled at the surface by a fine net.

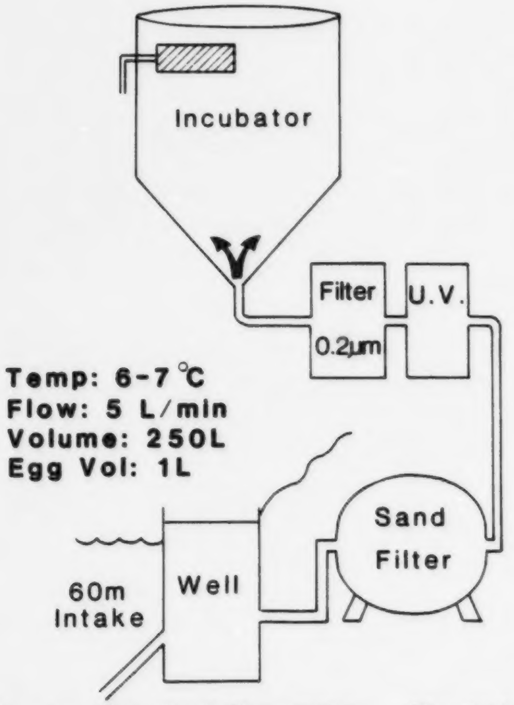


**Fig. 1. Egg incubation system. The tanks contain 250 L and have a capacity of about 50,000 eggs. Water intake is at 60 m depth. The water is filtered through 0.2-µm filters and UV-treated before introduction to the egg unit.**

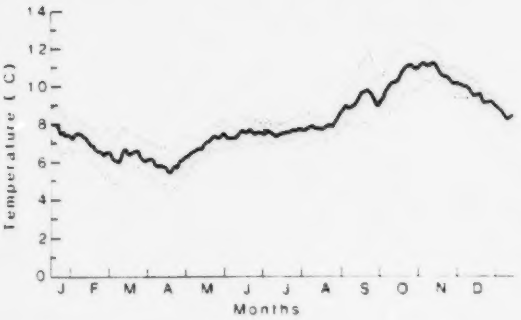


**Fig. 2. Seawater temperature at 55 m depth during a 12-mo cycle. Standard deviation shown.**

The larval rearing units consisted of conically shaped silos supplied with the same upwelling water system as the egg incubators (Fig. 3). The water supply to the silos originated from an inlet located at 60 m depth. The water was pumped through sand filters with an effective cutoff of about 20 µm. Before introduction to the silos, the water was temperature adjusted in a heat pump to give a final temperature of 6°C. The conically shaped silos served two purposes, to enhance the collection of dead material in the bottom of the silo, and to set up a water velocity gradient decreasing in speed as the water ascends in the tank. Tending of the silos was carried out in a similar manner as the egg incubator system. The water velocity gradient has proved to be efficient in keeping the larvae evenly distributed in the silo. Since there is variation in individual larval density within a batch of larvae, the gradient offers lift to each larva during the period of negative buoyancy around day 10 after hatching.

Live feed was introduced to the silos through a tube from the surface, terminating in the bottom water inlet. This provided an

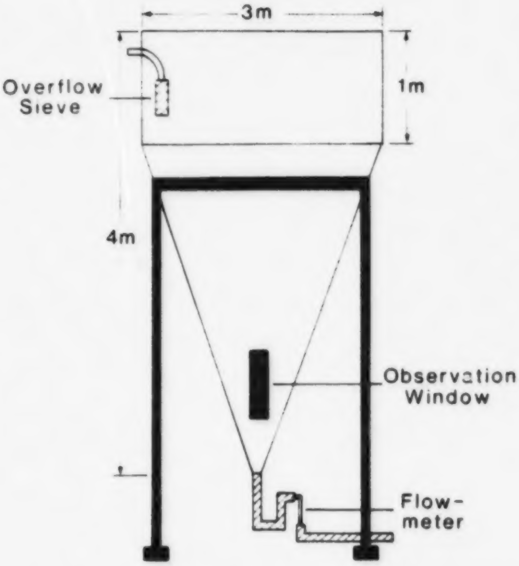


**Fig. 3. Larval rearing unit, the "silo." The unit receives temperature-adjusted water from a heat pump. Flow is regulated by separate flow meters on the inlet to the tank.**

adequate distribution of food organisms in the water column.

The light sources were vertically submerged light tubes with provision for dimming. The experiments included several light cycles from 24 h day to 12 h day.

## LIVE FEED PRODUCTION

Several species of live food organisms were tested in the feeding experiments. Alage (*Isochrysis galbana* tahiti str., *Tetraselmis suecica, Chaetoceros calcitrans* and *C. gracilis*) were also cultivated as enrichment feed for the rotifer *Brachionus plicatilis*, the brine shrimp *Artemia salina* and the harpacticoid copepod *Tisbe holothuriae*.

## ALGAE

The algae were grown in conical, 500-L tanks or in 100-L plastic bags. Use of internal light (light tubes inserted in water-proof plexiglass tubes) has proven more effective than external light. Batch cultures were grown in the plastic bags and semi-continuous cultures in the large tanks. Cultures were maintained semi-continuously for several months.

## ROTIFERS

Rotifers were grown in 250-L tanks at 18-20°C. Densities were kept at 200-300 individuals per mL. This density has proved to give a more stable system than both higher and lower densities (Lubzens 1984).

Different food regimes have been tested. The use of algae or yeast/cod liver oil has been tried, and also the combination of both. The benefit of a certain amount of algae in addition to the yeast/oil is evident. A rotifer culture started on clear seawater alone and fed yeast/oil will rapidly get infected by bacteria and ciliates and, therefore, be less suited for rapid growth of the rotifers. The addition of alage to the system had a positive effect on the environment in the tank, and also had a favorable effect on fatty acid supply for the rotifers and the fish larvae.

## *ARTEMIA*

The *Artemia* were hatched from commercially available resting eggs. Both San Francisco Bay Brand and Artemia Systems cysts were used. Decapsulation of the cysts using hypochloride (NaOCl) and sodium hydroxide (NaOH) improved the hatchability of the cysts (Sorgeloos et al. 1977) and was carried out routinely.

The cysts were hatched in conical 80-L tanks with a stocking density up to 2 g cysts/L and salinity of 15 ppt. Hatching was complete after 24 h at a temperature of 28°C. After hatching, the nauplii were transferred to seawater. When the nauplii reach instar II, they are able to feed, usually 48 h after hatching. Several different enrichment diets have been tried (Frippak, Selco, Superselco, capelin or cod roe based diets and emulsified marine oils).

## *TISBE*

*Tisbe* were grown in 200-L tanks with flat bottoms and/or immersed walls to increase the area for grazing. In contrast with *Acartia* sp. where the eggs are siphoned from the bottom, *Tisbe* carry their eggs until hatching. This made it more difficult to harvest the nauplii. Usually, parts of or the whole volume was siphoned through an adequate mesh and the adults were transferred back into the tank.

## RESULTS

The production of eggs from the broodstock has shown increasing results, both in volume and quality, during the last years. The quality of the eggs is evaluated from percent fertilization and survival through egg stage (Table 1). Survival of larvae through the yolk-sac stage is shown in Fig. 4.

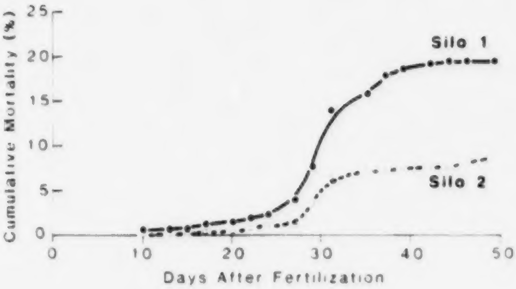


**Fig. 4. Larval mortality during yolk-sac stage in silos. The two graphs represent mortality in two similar silos containing larvae of different origin.**

Normally, the mortality in the silos peaks about 14 d after hatching or 25 d after fertilization. The cuase is unknown.

The number of fry that survived beyond metamorphosis in the intensive systems is less than 100. Results from start feeding experiments of halibut larvae are therefore difficult to interpret.

## DISCUSSION

The discussion of intensive fry production of Atlantic halibut easily leads to speculation as to why and when things went wrong. If no or few larvae survive beyond metamorphosis, the working hypothesis has to include all stages of production from broodstock management to food quality. The successful rearing of halibut in extensive systems has given the needed proof that fry production of Atlantic halibut is possible. The problem of egg quality in halibut farming seems to be a major factor in the process of rearing. Very little is known about the factors involved in the process of producing a high quality egg but, beyond any doubt, overripening is a dominant factor in decreasing egg quality (Kjørsvik et al. 1990). Halibut, like the rest of the flatfish, is a batch spawner. This implies that a small portion of eggs is released into the gonadal cavity at more or less regular intervals. The time between ovulation and intraovarian overripening starts has been shown to be very short. Therefore, the time of stripping has to be carefully calculated. Large, unexpected mortalities during the egg stage are most probably due to overripening, although this still needs closer investigation.

The yolk-sac stage has for a long time been considered to be the most critical event in halibut farming. Experiments to find adequate rearing units combined with the right environmental factors have been carried out. So far, the investigations have produced a method that will result routinely in about 50-60% survival through the yolk-sac stage. However, this percentage can be significantly increased if the problem of larval deformities is solved. In the production experiments conducted at the Aquaculture Station Austevoll, deformity rates of 80-90% have been observed. The reason behind these high rates of deformities is not known although several possibilities have been suggested (Pittman et al. 1987).

Light conditions in the silos during first feeding are probably one of the most critical factors in order to bring about feeding and growth in intensive units.

Intensive startfeeding of halibut has, so far, not been successful. From observations in the feeding units, it can be concluded that larval behavior was adequate in terms of feeding activity during the first few days after introduction of food. However, after a few days, the larvae terminated feeding and sought the darker areas of the silo. It is not known whether this was in response to the light or just a manifestation of poor food quality. Although some progress has been made in research to find ways to improve the technology for first feeding of halibut larvae, much remains to be done.

## REFERENCES

Blaxter, J. H. S., D. Danielsen, E. Moksnes, and V. Øyestad. 1983. Description of the early development of the halibut (*Hippoglossus hippoglossus* L.) and attempts to rear the larvae past first feeding. Mar. Biol. 73: 99-107.

Griffiths, D. 1975. Prey availability and the food of predators. Ecology 56: 1209-1214.

Haug, T., E. Kjørsvik, and P. Solemdal. 1984. Vertical distribution of Atlantic halibut (*Hippoglossus hippoglossus* L.) eggs. Can. J. Fish. Aquat. Sci. 41: 798-804.

Kjørsvik, E., A. Mangor-Jensen, and I. Holmefjord. 1990. Egg quality in fishes. Adv. Mar. Biol. 26: 71-113.

Leger, P., D. A. Bengtson, K. L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev. 24: 521-623.

Lubzens, E. 1984. Raising rotifers for use in aquaculture. Hydrobiologia 147: 245-255.

Pittman, K., L. Berg, and K. Naas. 1987. Morphological development of halibut (*Hippoglossus hippoglossus*) larvae with special reference to mouth development and metamorphosis. Int. Counc. Explor. Sea C.M. 1987/F:18.

Rabben, H. 1987. A stripping method for Atlantic halibut (*Hippoglossus hippoglossus* L.). Int. Counc. Explor. Sea C.M. 1987/F:40.

Rabben, H., T. O. Nilsen, I. Huse, and A. Jelmert. 1986. Production experiment of halibut fry in large enclosed water columns. Int. Counc. Explor. Sea C.M. 1986/F:19.

Rollefsen, G. 1934. The eggs and larvae of the halibut (*Hippoglossus hippoglossus*). K. Norske Vitensk. Selsk. Skr. 7: 20-23.

Solemdal, P., S. Tilseth, and V. Øyestad. 1974. Rearing of halibut. I. Incubation and the early larval stages. Int. Counc. Explor. Sea C.M. 1974/F:41.

Sorgeloos, P., E. Bossuyt, E. Lavina, M. Baeza-Mesa, and G. Personne. 1977. Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. Aquaculture 12: 311-316.

Watanabe, T., T. Tamiya, A. Oka, M. Hirata, C. Kitajima, and S. Fujita. 1983. Improvement of dietary value of live foods for fish larvae by feeding them on n-3 highly unsaturated fatty acids and fat-soluble vitamins. Bull. Jap. Soc. Sci. Fish. 49: 471-479.

Table 1. Data from the halibut hatchery, 1989.

| Batch no. | Date | Female no. | Percent fertilized | Diameter (mm) | SD | Mortality (%) |
|---|---|---|---|---|---|---|
| 1 | 10 Feb | 3 | 44 | 3.00 | 0.15 | 1.40 |
| 2 | 13 Feb | 3 | 16 | 3.08 | 0.05 | 1.00 |
| 3 | 16 Feb | 17 | 90 | 3.10 | 0.04 | 0.32 |
| 4 | 19 Feb | 3 | 10 | 3.02 |  | 1.40 |
| 5 | 20 Feb | 4 | 60 | 3.10 | 0.03 | 1.30 |
| 6 | 22 Feb | 17 | 70 |  |  | 0.10 |
| 8 | 23 Feb | 3 | 37 |  |  | 0.60 |
| 9 | 25 Feb | 17 | 86 | 3.10 | 0.06 | 0.10 |
| 10 | 26 Feb | 4 | 89 | 3.17 | 0.06 | 0.28 |
| 11 | 28 Feb | 17 | 96 | 3.05 | 0.05 | 0.03 |
| 12 | 28 Feb | 17 | 71 | 3.03 | 0.04 | 0.13 |
| 13 | 01 Mar | 4 | 73 | 3.10 | 0.05 | 0.20 |
| 14 | 03 Mar | 3 | 39 | 3.00 | 0.06 | 0.65 |
| 15 | 03 Mar | 17 |  | 3.02 | 0.05 |  |
| 16 | 03 Mar | 9 | 51 | 3.13 | 0.05 | 0.10 |
| 17 | 04 Mar | 4 | 80 | 3.08 | 0.04 | 0.40 |
| 18 | 06 Mar | 17 | 95 | 3.04 | 0.036 | 0.34 |
| 19 | 06 Mar | 9 | 95 | 3.03 | 0.08 | 0.24 |
| 20 | 08 Mar | 4 | 98 | 3.13 |  |  |
| 21 | 10 Mar | 9 |  | 3.02 | 0.06 | 0.12 |
| 22 | 11 Mar | 4 | 54 | 3.03 | 0.062 | 0.60 |
| 23 | 13 Mar | 5 | 31 | 3.17 | 0.046 | 1.30 |
| 24 | 13 Mar | 9 | 69 | 3.03 | 0.705 | 0.20 |
| 25 | 13 Mar | 9 | 97 | 3.09 | 0.815 | 0.10 |
| 26 | 13 Mar | 4 | 43 | 3.13 | 0.562 | 0.45 |
| 27 | 16 Mar | 9 | 62 | 3.04 | 0.053 | 0.52 |
| 28 | 17 Mar | 4 | 66 | 2.943 | 0.062 | 0.10 |
| 29 | 17 Mar | 49 | 4 |  |  |  |
| 30 | 19 Mar | 5 | 84 | 3.08 | 0.058 | 0.66 |
| 31 | 20 Mar | 9 | 90 | 3.04 | 0.052 |  |
| 32 | 22 Mar | 5 | 88 | 3.00 | 0.055 | 0.57 |
| 33 | 23 Mar | 9 | 42 | 2.99 | 0.083 | 0.33 |
| 34 | 26 Mar | 5 | 85 | 3.07 | 0.086 | 0.66 |
| 35 | 27 Mar | 9 | 90 | 3.00 | 0.066 | 0.23 |
| 36 | 29 Mar | 5 | 98 | 3.10 | 0.04 | 0.26 |
| 37 | 30 Mar | 9 | 95 | 2.99 | 0.046 | 0.38 |
| 38 | 02 Apr | 5 | 80 | 3.05 | 0.081 | 1.41 |
| 39 | 02 Apr | 9 | 89 | 3.03 | 0.081 | 0.13 |
| 40 | 05 Apr | 5 | 64 | 3.01 | 0.062 | 1.63 |
| 41 | 05 Apr | 49 | 30 | 2.91 | 0.059 | 0.31 |
| 42 | 05 Apr | 9 | 94 | 3.00 | 0.041 | 0.30 |
| 43 | 06 Apr | 5 | 39 | 3.13 | 0.053 | 0.20 |
| 44 | 06 Apr | 9 | 76 | 2.97 | 0.036 |  |
| 45 | 08 Apr | 5 | 93 | 3.07 | 0.068 | 0.45 |
| 46 | 08 Apr | 9 | 95 | 2.98 | 0.033 | 0.43 |
| 47 | 10 Apr | 49 | 38 | 2.87 | 0.057 | 0.30 |
| 48 | 10 Apr | 5 | 88 | 3.08 | 0.057 | 0.52 |
| 49 | 11 Apr | 5 | 88 | 3.08 | 0.047 | 0.27 |
| 50 | 13 Apr | 5 | 41 |  |  | 0.11 |
| 51 | 13 Apr | 49 | 90 | 2.82 | 0.033 | 0.20 |

# THE ATLANTIC HALIBUT YOLK SAC LARVA: A SUMMARY OF THE RESULTS FROM FOUR YEARS OF EXPERIMENTATION

Karin Pittman
Department of Fisheries Biology
University of Bergen
P. O. Box 1839 Nordnes
Bergen, Norway

## ABSTRACT

Pittman, K. 1990. The Atlantic halibut yolk sac larva: a summary of the results from four years of experimentation, p. 161-168. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

The developmental behavior and morphology of the yolk sac Atlantic halibut larva has been followed from hatching to beyond metamorphosis. Temperatures near 4°C seem to give best growth, yolk absorption efficiency, and normal development. High rates of mortality were observed early in the development and may also be correlated with high flow rates, but have not been directly correlated with bacteria. Biochemical, histological, morphological, and behavior data point to 20-30 d posthatching as the most likely period for initiation of exogenous food uptake, even though this is only half way through the yolk sac stage. Preliminary results from a temperature experiment involving eggs and yolk sac stages indicate that relative protein synthesis at hatching was better when the eggs were incubated at 3°C than at either 6° or 9°C. The presence or absence of Kuppfers vesicles in the developing embryo may be a possible indicator of embryo quality.

## RÉSUMÉ

Pittman, K. 1990. The Atlantic halibut yolk sac larva: a summary of the results from four years of experimentation, p. 161-168. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

On a étudié le comportement de développement ainsi que la morphologie des larves embryonnaires du flétan de l'Atlantique, à partir de l'écloserie jusqu'après la métamorphose. Les températures qui se situent autour des 4°C semblent donner un meilleur taux de croissance, une meilleure résorption du sac vitellin et un développement plus normal. On a observé un taux de mortalité élevé au début du développement ce qui pourrait être associé à un fort débit; mais on n'a pas encore établi de lien direct entre cette mortalité et la présence de bactéries. Les données biochimiques, histologiques, morphologiques ainsi que les données sur le comportement semblent indiquer que la période de 20 à 30 jours qui suit l'écloserie est la meilleure période pour commencer l'alimentation exogène, même si ce n'est que la moitié de l'étape du sac vitellin. Les résultats préliminaires d'une expérience thermique mettant en cause l'étape des oeufs et l'étape du sac vitellin indiquent que la synthèse des protéines lors de l'écloserie est meilleur lorsque l'incubation des oeufs se fait à 3°C plutôt qu'à 6°C ou 9°C. La présence ou l'absence des vésicules de Kupffer dans l'embryon en développement pourrait être indicatif de la qualité de l'embryon.

## INTRODUCTION

There has been only one halibut (*Hippoglossus hippoglossus*) larva caught *in situ* in the northeast Atlantic (Haug et al. 1989), so our knowledge of the larval stage of this species is truly limited. Observations gathered during the rearing experiments at Austevoll Aquaculture Station and at Akvaforsk in Sunndalsøra have allowed us to describe the development of behavior and morphology from hatching to metamorphosis and to determine some of the effects of temperature and light on the larvae.

The following is a brief description of the development of the larvae and the results of experimental manipulation so far, mostly involving temperature. The preliminary results of a thermal experiment on eggs, larvae, and bacteria are also briefly presented.

## EFFECTS OF TEMPERATURE ON EGGS AND YOLK SAC LARVAE

Although an optimal temperature for eggs and larvae of halibut has not yet been defined, there is a growing body of evidence that this probably lies between 3 and 6°C. An experiment was conducted this year (1989) to study the thermal effects on egg and yolk sac stages. Differences in rates of development were apparent from the time required for first cell divisions at various temperatures (Table 1). Those embryos incubated at 3°C had larger yolk sacs and higher relative protein synthesis (RNA/DNA) when hatched than did embryos incubated at 6 or 9°C (Fig. 1, 2), and there was a difference in standard length at hatching (Bergh et al. 1989). Preliminary results have also indicated that eggs incubated at 9°C had developed further (DNA, protein, yolk absorption) at the point of hatching than had the others incubated at lower temperatures. For further evaluation of the data regarding egg and larval development and statistical analyses, the reader is referred to Pittman et al. (1990).

An interesting observation was the timing of appearance and quantity of Kuppfers vesicles, small bladders of unknown function which have been observed posterior to the yolk sac in embryos and newly hatched larvae since these were first described by Rollefsen (1934). These appeared just prior to the closing of the blastopore in all thermal groups; the mean number per embryo was 5 (9°C), 0.3 (6°C), and 1.3 (3°C). Many in the 6 and 3°C groups had no vesicles (Bergh et al. 1989). Kjørsvik has found these vesicles appear as indeterminate "unfinished" structures in histological preparations of other groups of halibut larvae (pers. commun.). In our groups, it also seemed that the nature of the vesicles was different at

Table 1. Egg development (in hours) from fertilization to hatching in halibut eggs incubated at 3, 6, and 9°C. Three incubators (a,b,c) were used at each temperature.

| Stage | 3a | 3b | 3c | 6a | 6b | 6c | 9a | 9b | 9c |
|---|---|---|---|---|---|---|---|---|---|
| 2-cell | 8.1 | 8.8 | 8.8 | 6.8 | 6.8 | 6.8 | 4.3 | 5.5 | 5.1 |
| 4-cell | 13.6 | 13.9 | 13.9 | 9.3 | 10.8 | 10.8 | 6.8 | 7.8 | 7.5 |
| 8-cell | 17.9 | 19.6 | 19.9 | 12.6 | 13.6 | 13.6 | 8.4 | 9.3 | 9.3 |
| 16-cell | 24.6 | 23.5 | 24.1 | 15.6 | 16.8 | 16.8 | 10.8 | 11.8 | 11.8 |
| 32-cell | 28.6 | 28.6 | 28.6 | - | - | - | 12.6 | 13.6 | 13.6 |
| Germring formed | 125 | 118 | 125 | 74 | 74 | 74 | 49.5 | 49.5 | 49.5 |
| Blastopore closing | 330 | 295 | 295 | 144 | 145 | 145 | 103 | 106 | 107 |
| Heartbeat | 536 | 524 | 536 | 297 | 297 | 297 | 191 | 191 | 191 |
| 50% hatching | 600 | 600 | 600 | 336 | 336 | 336 | 211 | 211 | 211 |

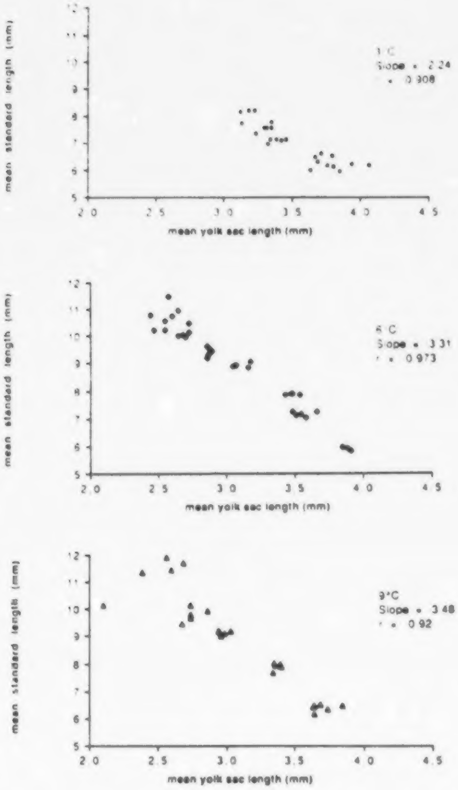


**Fig. 1. Mean standard length vs mean yolk sac size of halibut larvae incubated at 3, 6 and 9°C from fertilization onward (from Bergh et al. 1989).**

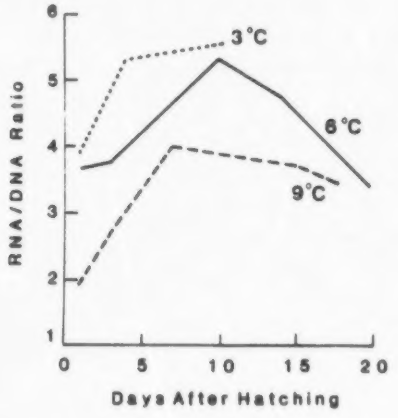


**Fig. 2. RNA/DNA ratios of halibut larvae incubated at 3, 6, and 9°C from fertilization onward (from Bergh et al. 1989).**

the different temperatures. This has led us to question whether the vesicles are an artifact due to (suboptimal) abiotic factors or their presence may be an indicator of egg quality. Samples have been taken for histological examination.

A comparison of development rates at various temperatures after hatching is summarized in Fig. 4, where 4a shows the results when temperatures were changed 1 wk after hatching and 4b shows the results when eggs and larvae were subject to continuous temperature regimes. Size at end-of-yolk-sac was greatest at a mean larval incubation temperatures of 4°C (Fig. 3; Pittman et al. 1989) while deformities such as gaping and oedema were most common at 9°C and higher (Bolla and Holmefjord 1988; Pittman et al. 1989). Fewer day degrees were needed to reach end-of-yolk-sac in cold water than in warm water (25% less) and the use of day degrees in determining development has been dropped by some investigators.

## DEVELOPMENT OF THE YOLK SAC LARVA

During the approximately 50 d from hatching to end-of-yolk-sac, the halibut larva changes from a primitive, passive, and unpigmented yolk-sac larva to an actively swimming predator. The larva emerges from the egg when the ring of hatching enzymes has broken down the zona radiata in a circle around the region of the larval head. This allows a cap of shell to be pushed out from within, the larva escaping through the hold (Helvik 1988). When hatched at 6-7 mm standard length, the larva is completely unpigmented and the head is con-

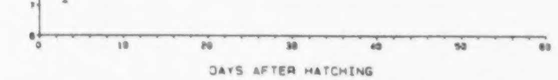


**Fig. 3. Mean standard length from hatching to end-of-yolk-sac for halibut larvae incubated at 4, 6, and 9°C from 1 wk after hatching. Vertical lines are standard deviation and the final point for the 4° group is only one measurement (from Pittman et al. 1989).**

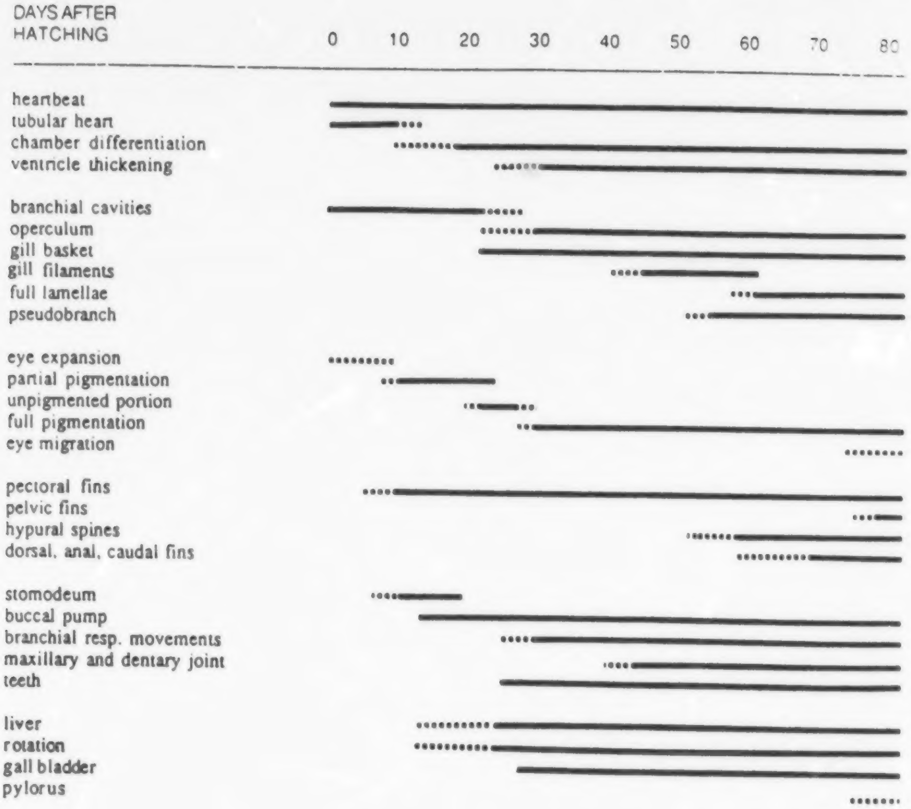


**Fig. 4a. The development of halibut larvae from eggs incubated at variable temperatures and given temperatures between 4 and 7°C from hatching to metamorphosis. These larvae experienced variable thermal conditions within the rearing units and thus the imprecise determination of the timing of some developmental events is reflected in the broken lines (from Pittman et al. 1990).**

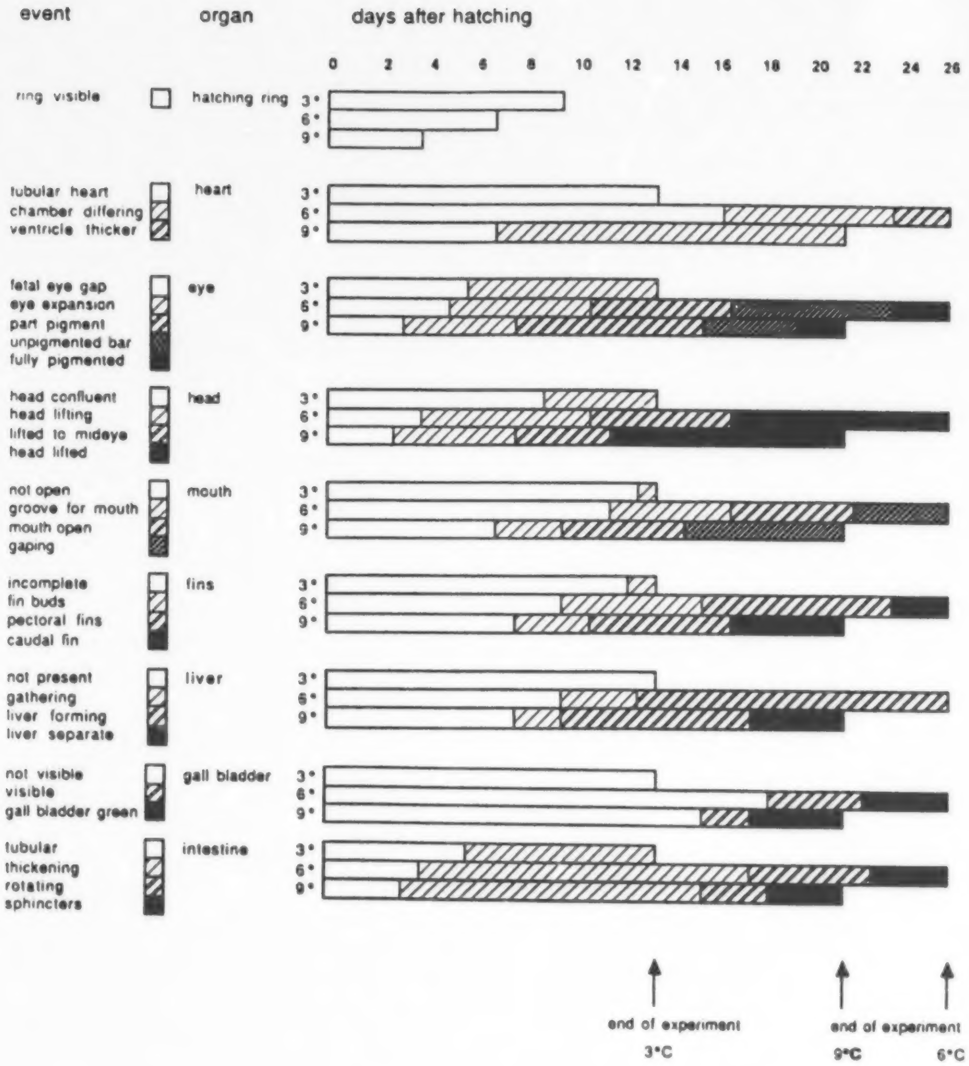


**Fig. 4b. The development of halibut larvae when eggs and larval stages are incubated at constant temperatures (3, 6, or 9°C) from fertilization to the end of the experiment about half way through the yolk-sac stage (from Bergh et al. 1989).**

fluent with the yolk sac (Rollefsen 1934), there is no stomodeum (Pittman et al. 1989), and a faint ring of hatching glands can be seen on the anterior yolk sac reaching to behind the otoliths. There is a pair of external branchial pits (Pittman et al. 1989) connected to the esophagus (Kjørsvik, pers. commun.) through which the larva may drink shortly after hatching (Tytler and Blexter 1988).

Data from 4 yr of halibut production have contributed to a description of the developing larva. A proposal has been forwarded to divide the yolk sac phase into four stages, according to organogenesis and behavior (Pittman et al. 1990). In all stages, the notochord remains straight and the eyes symmetrical. Growth is mostly in standard length rather than myotome height until the onset of exogenous feeding.

**Stage 1.** Yolk present; notochord straight; no stomodeum (mouth); no chromatophores; eyes symmetrical and pressed against the yolk; eye gap visible; a pair of external branchial pits are posterior to the head; intestine straight; no pectoral fins; simple heart; body generally lies passive and almost vertical in the water with head downwards; infrequent swimming generally in the vertical plane, mean speed 0.5 mm/s; passive rising in the water column the first few days, thereafter sinking.

**Stage 2.** Yolk present, yolk sac changing from an elliptical form to a pear-shaped form; notochord straight; mouth present as a small opening; eyes symmetrical, circular, and variously pigmented with an unpigmented dorsal gap; branchial pits expand to short opercular flaps; intestine thickening and beginning to loop; pectoral fins develop from buds to paddle-like fins; heart differentiating into four chambers; liver and gall bladder develop; gall bladder may be colored light green for a short period; body nearly horizontal in water; mean swimming speed increases from 0.5 to 3.0 mm/s when active; generally passive sinking.

**Stage 3.** Yolk present; yolk sac reducing to tubular form on right side of intestine, connected to medial side of liver; notochord straight; mouth becomes functional; eyes symmetrical and fully pigmented; gill basket developing with some filaments on the arches; intestine fully looped; rectal sphincter develops; ventricular cardiac muscles thicken; mean length of active periods increases from about 5 s to over 1 min; swimming more often horizontally, mean swimming speed between 2-3 mm/s; generally passive rising; larvae can assume "C" and "S" forms in the horizontal plane for a few seconds at a time.

**Stage 4.** Little yolk left; notochord straight; hypural thickening; hypural spines visible; mouth functional, buccal valve billows out when buccal cavity contracts; eyes symmetrical; gill filaments on arches; some black melanophores along margin of ventral finfold and notochord; ratio of standard length to myotome height is still greater than 15; larva displays searching activity and attacks prey items but mean duration of active periods drops; may coincide with the onset of exogenous feeding.

Stages 1 and 4 are of short duration (less than a week, depending on temperature) whereas the bulk of the larval period is made up of stages 2 and 3. Premature gaping or "lockjaw" appears during stage 2 and seems to be associated with higher than optimal temperatures (Bolla and Holmefjord 1988; Pittman et al. 1989). Oedema will cause the afflicted larvae to float near the surface during stage 3 and is also correlated with too high temperatures (Pittman et al. 1989). Potential prey items may be added during stages 3-4, which may coincide with first feeding, but larvae in these stages which have not fed are distinguished by the lack of growth in myotome height.

The timing of the appearance of the gill basket and hypural fin rays may be inaccurate. A preliminary test of differential staining for bone and cartilage was carried out on a few larvae which had been preserved in formalin for about 10 mo (Methven and Pittman, unpubl. data). Positive staining for cartilage was found on some larvae within a month after hatching and the fin rays could be clearly distinguished. The first areas to stain positively for bone were the tip of the dentary and maxillary where the minute teeth were anchored after the onset of exogenous feeding. Four pharyngeal teeth, located immediately anterior to the esophagus, were found on a stained larva about 40 d after hatching at 9°C.

## TIME OF FIRST FEEDING

A crossroads seems to be reached at about 25-30 d after hatching, when activity increases, yolk supply diminishes, and the necessary digestive organs have developed. Recent data (Bergh et al. 1989) show that the relative protein synthesis in larvae returns to the level found at hatching at about 25-30 d after hatching. Histologically, the intestine seems to be sufficiently developed at about 25 d after hatching (Kjørsvik, pers. commun.). Since standard lengths of larvae raised at 4°C exceed those of larvae raised at 6 and 9°C at this time (Pittman et al. 1989), it is proposed that energy becomes limiting during the second half of the yolk sac stage. Some researchers have recorded expgenous feeding as early as 23 d after hatching (Berg, unpubl. data, cited in Pittman et al. 1990). Thus, it seems that the halibut larva is capable of, and may already require, exogenous food uptake 25-30 d after hatching.

## MORTALITIES

Most rearing experiments since 1986 have experienced an increase in larval mortality between days 10-15, although the cause was unknown. Some light has been shed on this uncertainty by this recent temperature experiment (Bergh et al. 1989), where the increase in mortality occurred at the same stage in development at 6 and 9°C in all incubators (about days 12 and 8, respectively). The mortality preceded a rise in free-living bacteria which in turn preceded a rise in ammonia. Thus, it seems that a vicious circle commences, where increased ammonia levels further weaken remaining larvae which leads to an increase in bacteria which further increases ammonia levels, etc.

However, not all rearing systems or rearing experiments experience the same pattern of mortality (Lien, pers. commun.). Flow has been implicated as a cause (Opstad and Bergh, unpubl. data) where flow rates above one full water exchange per day gave increased mortalities and were correlated to the deterioration of the tissue on the snout and fins (Bergh et al. 1989).

## CONCLUSIONS

It seems that the environment experienced by the egg stage affects the quality and growth potential of the emergent larva. The larva may also be affected by temperature during the yolk-sac stage and be subject to sublethal deformities which allow it to survive until yolk exhaustion but will impair prey capture ability. Best growth and survival has been obtained at low temperatures (about 4°C) and at low rates of water exchange (about one full exchange per day). Bacteria have not yet been implicated as a primary cause of mortality, although it seems that a vicious circle exists where larval mortality leads to increased bacteria which leads to increased ammonia which weakens the remaining larvae, etc.

Further examination will need greater control over temperature, flow, and hygiene at both the egg and larval stages. Behavioral aspects, particularly regarding the effects of various wavelengths and brightnesses of light on search and capture of prey, need to be systematically investigated. Current practices refrain from giving the larvae daylight until (nearly) full eye pigmentation, although the effects of this timing on neural development and visual acuity have not been examined. Identifying optimal values of other parameters such as salinity and oxygen may not only aid in raising greater numbers of Atlantic halibut larvae to viable fry, but also aid in determining the location of the larvae in the wild.

## REFERENCES

Bergh, Ø., I. Opstad, K. Pittman, A. B. Skiftesvik, L. Skjolddal, H. Strand, and V. Vanthuyne. 1989. Preliminary report on the effects of temperature on the development of eggs and larvae of halibut (*Hippoglossus hippoglossus*) and on the bacterial population in the incubators. Intern. Counc. Explor. Sea C.M./F:19 (mimeo).

Bolla, S., and I. Holmefjord. 1988. Effect of temperature and light on development of Atlantic halibut larvae. Aquaculture 74: 355-358.

Haug, T., E. Kjørsvik, and K. Pittman. 1989. Observations on a wild Atlantic halibut larva, *Hippoglossus hippoglossus* (L.). J. Fish Biol. 34: 799-801.

Helvik, J. V. 1988. Klekkeprosessen hos kveite (*Hippoglossus hippoglossus*) (The hatching process in halibut). Masters Thesis, Dept. Fish. Biol., Univ. Bergen, 87 p.

Pittman, K., Ø. Bergh, I. Opstad, A. B. Skiftesvik, L. Skjolddal, and H. Strand. 1990. Development of eggs and yolk sac larvae of halibut (*Hippoglossus hippoglossus* L.) at 3, 6 and 9°C. J. Appl. Ichthyol. (in press).

Pittman, K., A. B. Skiftesvik, and L. Berg. 1990. Morphological and behavioural development of halibut (*Hippoglossus*

*hippoglossus*) larvae. J. Fish Biol. (in press).

Pittman, K., A. B. Skiftesvik, and T. Harboe. 1989. Effect of temperature on growth rates and organogenesis in the yolk sac larvae of halibut (*Hippoglossus hippoglossus* L.). Rapp. P.-v. Réun. Cons. Int. Explor. Mer 191: 421-430.

Rollefsen, G. 1934. The eggs and larvae of the halibut (*Hippoglossus vulgaris*). Kongl. Norske Vidensk. Selsk. Forh. nr. 7.

Tytler, P., and J. H. S. Blaxter. 1988. Drinking in yolk sac stage larvae of the halibut, *Hippoglossus hippoglossus* (L.). J. Fish Biol. 32: 493-494.

# RESPONSE OF PLASMA SODIUM, CHLORIDE AND OSMOTIC CONCENTRATIONS IN ATLANTIC COD (*GADUS MORHUA*) FOLLOWING DIRECT TRANSFER TO DILUTED SEAWATER

by

J.-D. Dutil[1], M. Besner[2], and J. Munro[1]

[1]Ministère des Pêches et des Océans, 850 route de la Mer,
C.P. 1000, Mont-Joli, Québec.
[2]Université du Québec à Rimouski, 300 allée des Ursulines,
Rimouski, Québec.

## ABSTRACT

Dutil, J.-D., M. Besner, and J. Munro. 1990. Response of plasma sodium, chloride and osmotic concentrations in Atlantic cod (*Gadus morhua*) following direct transfer to diluted seawater, p. 169-174. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Atlantic cod were directly transferred to low salinities (0, 7, 14 and 21 g/L from 26-28 g/L). No cod survived when directly transferred to fresh water, but no mortality occurred in brackish waters. Significant differences were observed between transfers made in December (water temperature: 4.4-5.4°C) and February (water temperature 1.2-2.5°C). Within each month, ionic and osmotic concentrations 28 d from transfer were highest in cod held in seawater (26-28 g/L control) and lowest in cod held at 7 g/L. The latter treatment resulted in a longer period to acclimate than at 14 or 21 g/L, particularly in February. Nevertheless, a plateau was eventually reached, indicating that cod exposed to 7 g/L salinity would withstand such conditions for prolonged periods of time, suggesting that cod rearing under estuarine conditions may be feasible.

## RÉSUMÉ

Dutil, J.-D., M. Besner, and J. Munro. 1990. Response of plasma sodium, chloride and osmotic concentrations in Atlantic cod (*Gadus morhua*) following direct transfer to diluted seawater, p. 169-174. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

La morue de l'Atlantique a été transférée directement dans des eaux à faible salinité (à partir de 26-28 g/L à 0, 7, 14 et 21 g/L). Aucune morue n'a survécu lorsque transférée directement en eau douce, mais aucune mortalité n'a été enregistrée en eau saumâtre. On a observé des différences considérables entre les transferts qui ont eu lieu en décembre (température de l'eau: 4,4-5,4°C) et ceux qui sont survenus en février (température de l'eau: 1,2-2,5°C). Pour chaque mois, les concentrations ioniques et osmotiques 28 d après le transfert des groupes étaient les plus fortes pour la morue dans l'eau de mer (groupe témoin à 26-28 g/L) et les plus faibles pour la morue à 7 g/L. Celle-ci a mis plus de temps à s'acclimater à son milieu que la morue à 14 ou 21 g/L, surtout en février. Néanmoins, on a par la suite atteint un plateau, ce qui indique que la morue exposée à une salinité de 7 g/L supporterait ces conditions pendant des périodes prolongées et, donc, que l'on pourrait faire l'élevage de la morue en milieu estuarien.

## INTRODUCTION

Salmonids among freshwater species have drawn considerable attention, both as an interesting model of ontogenic changes in osmoregulatory capacity, i.e. the parr-smolt transformation, and for practical purposes, i.e. to determine how and when smolts should be transferred from fresh water to marine rearing pens in order to insure minimal losses and better growth in aquaculture facilities.

Though there has been numerous studies on the subject of ionic and osmotic regulation in anadromous and catadromous species of fish, there appears to be little information on the capacity of marine fishes to tolerate and adapt to fresh and brackish waters. Marine fishes are generally considered strictly stenohaline and the question of their tolerance to hypo-osmotic water is rarely addressed. Thirteen fish species from Hong Kong were exposed to diluted seawater for a period of 2 wk (Wu and Woo 1983). The study included offshore deep-water species as well as coastal species. The former were slightly less tolerant to hypo-osmotic conditions, but no mortality was observed at salinities greater than 10 g/L, indicating that many marine fishes are more euryhaline than can be inferred from their life history. The ability of marine species to survive at low salinities suggests that rearing marine species under brackish conditions might have some potential.

This study originated from our desire to assess indigenous marine species as potential candidates for aquaculture in the estuary of the St. Lawrence River. Indeed, in the St. Lawrence, climatic conditions in winter prevent the expansion of salmonid culture in sea pens on a year-round basis due to low lethal temperatures (Saunders et al. 1975). Many indigenous marine species are more tolerant to low temperatures than are salmonids. Atlantic cod, for instance, tolerate very low temperatures; cod feed at low temperatures (Saunders 1963, and our own observations) and synthesize antifreeze glycoproteins in winter (Davies et al. 1988). However, the St. Lawrence estuary and sheltered locations on the coast of the Gulf of St. Lawrence are characterized by brackish conditions near the water surface. Surface salinities vary, for instance, with location in the estuary, freshwater runoff, and degree of mixing induced by tides and wind events. Marine fish aquaculture in such locations might be limited by the ability of those fish to withstand short-term changes in salinity. This study investigates the tolerance of Atlantic cod (*Gadus morhua*) to low salinity.

## MATERIALS AND METHODS

Five experiments were conducted over a period of 12 mo. Preliminary analyses of the first two experiments only will be presented in this short communication.

Atlantic cod were caught using bottom trawls at depths ranging from 30-40 m. They were kept in 3.7-m diameter holding tanks for 3 wk before starting the first experiment (December). Fish from the same group and similar average size were used 2 mo later for the second experiment (February). Mean lengths and mean weights were 42 ± 4 cm, 595 ± 215 g, and 42 ± 4 cm, 539 ± 188 g in December and February, respectively. Gonadosomatic ratio (weight of gonad/[body weight minus gonad weight]) increased slightly from 3.1-4.2% in males and from 1.5-2.6% in females between December and February.

### HOLDING CONDITIONS

Seawater was pumped from the St. Lawrence estuary to three mixing tanks where fresh, unchlorinated city water was added in order to obtain three different salinities: 7, 14, and 21 g/L. Water flowed from those tanks into three experimental tanks. A control tank received undiluted seawater. Photoperiod was adjusted to the natural seasonal cycle. Dissolved oxygen and salinity were monitored twice daily using a YSI model 57 oxygen meter and a YSI model 33 salinity meter. Salinity was also checked periodically with a Beckman induction salinometer (model RS-9).

Dissolved oxygen concentrations were maintained above 8.4 mg/L. Water temperature and salinity followed those of the natural environment. Seawater temperature decreased steadily in December while it remained fairly constant in February. Mean salinity and temperature values for December and February were as follows (mean ± SE):

| | | 7 | 14 | 21 | Control |
|---|---|---|---|---|---|
| Salinity | D | 7.0±.2 | 14.0±.1 | 20.7±.3 | 26.2±.4 |
| | F | 7.1±.1 | 14.1±.1 | 21.4±.2 | 28.7±.2 |
| Temperature | D | 5.4±.2 | 5.3±.3 | 4.8±.2 | 4.4±.2 |
| | F | 2.5±.1 | 2.3±.1 | 2.0±.1 | 1.2±.5 |

The salinity of our freshwater supply was in the range 0.2-0.3 g/L.

## SURVIVAL

Twenty cod were transferred directly into each of four experimental tanks (0, 7, 14, and 21 g/L salinity) and into one control tank. Survival was monitored over 96 h using a video camera and recorder.

## OSMOTIC ADAPTATION

Eighty cod were transferred directly to each tank. Ten cod were sampled from each tank after 1, 2, 3, 4, 7, 14, 21, and 28 d. The fish were killed by a blow to the head and their blood drawn from the caudal vein using ammonium-heparin coated syringes. Plasma sodium and chloride concentrations were measured using an ion analyzer (Corning, Model 644) and osmotic pressures were measured with a micro-osmometer (Advanced Instrument, Model 3 MO).

Statistical tests were performed only on plasma osmotic concentrations measured at the end of the experiments (day 28). A two-way analysis of variance was done using the date as factor 1 (two levels, December and February) and factor 2 was salinity (three levels, 14, 21, and control). Due to a lack of homogeneity in variances, data for the 7 g/L treatment was not included in the analysis of variance; the variability in osmotic concentrations in cod exposed to 7 g/L salinity in December was much lower than that of cod exposed to low salinities in February. Differences in osmotic concentrations between December and February for cod held in 7 g/L salinity were tested using Mann-Whitney's U-test. Tests were made *a posteriori* using the Student-Newman-Keuls multiple range test. Homogeneity of variances was tested using Hartley's $F_{max}$ test.

## RESULTS AND DISCUSSION

## SURVIVAL

No mortality occurred among cod exposed to brackish waters, including those held in 7 g/L salinity, that is, in hypo-osmotic conditions, both in December and February. However, no cod survived a direct transfer to fresh water. Fifty percent died within the first 2.5 h. In February, cod resisted freshwater exposure for a longer period. Half the fish survived 8 h, but they had all died after 17 h of exposure. Odense et al. (1966) acclimated 27 cod to low salinity by gradually decreasing the salinity in the tank over a 120-d period. They found that cod resisted to low salinity, but died when salinity dropped below 0.4% NaCl.

## OSMOTIC PERFORMANCE

Preliminary analyses suggest that the patterns of changes in ion and osmotic concentrations over time were similar for treated (7, 14, and 21 g/L salinity) and control animals in December. The plasma sodium concentration of cod held in lower salinities never departed markedly from the controls (26.2 g/L salinity) (Fig. 1). This was also true of cod held in 14 and 21 g/L salinity in February (Fig. 2). However, cod transferred to 7 g/L salinity in February showed a marked drop in plasma sodium concentrations over 2 wk and apparently had more difficulty establishing new equilibrium conditions. Nevertheless, they reached a plateau indicating that cod would be able to withstand such conditions for prolonged periods of time. This assertion is strengthened by our observation that the new concentrations reached are similar to those measured in cod held at 7 g/L in December (Fig. 3). Cod maintained at higher salinities in February (14 and 21 g/L), like the controls, did not exhibit such a decrease towards concentrations measured in cod held at the same salinities in December (Fig. 4, 5). Thus, osmoregulatory performance under hypo-osmotic conditions may be impaired at lower temperatures so that one cannot exclude the possibility that mortality could have occurred at temperatures below 2°C.

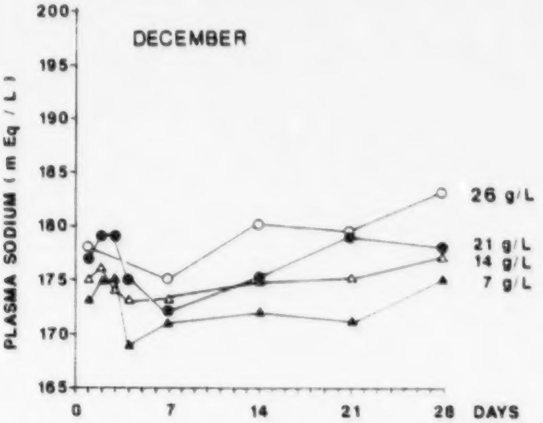


**Fig. 1. Temporal changes in plasma sodium concentrations in Atlantic cod transferred directly to waters of three different salinities in December. Controls were held in 26 g/L salinity. Temperature averaged 4.4°C (control) to 5.4°C (7 g/L) over the 28-d period.**

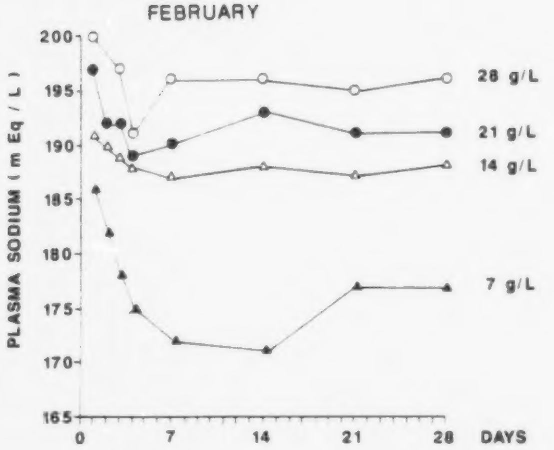


Fig. 2. Temporal changes in plasma sodium concentrations in Atlantic cod transferred directly to waters of three different salinities in February. Controls were held in 28 g/L salinity. Temperature averaged 1.2°C (control) to 2.5°C (7 g/L) over the 28-d period.

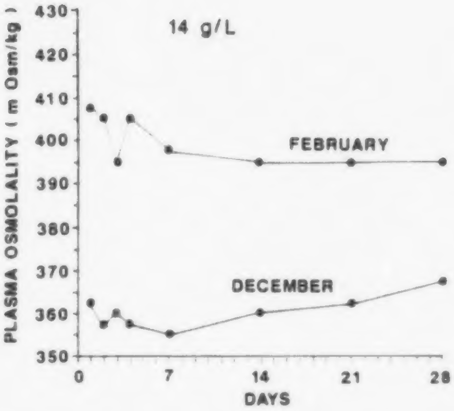


Fig. 4. Temporal changes in plasma osmolality in Atlantic cod transferred directly to brackish water at 14 g/L in December and February. Temperature averaged 5.3°C in December and 2.3°C in February

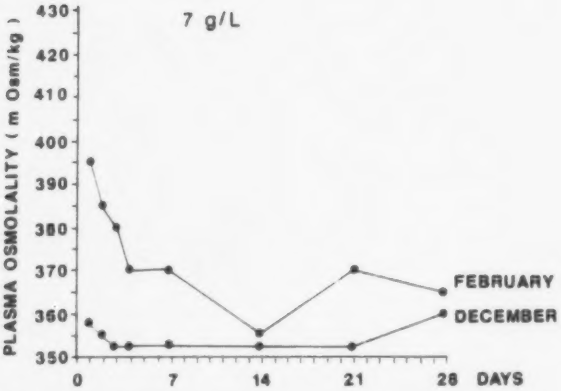


Fig. 3. Temporal changes in plasma osmolality in Atlantic cod transferred directly to brackish water at 7 g/L in December and February. Temperature averaged 5.4°C in December and 2.5°C in February.

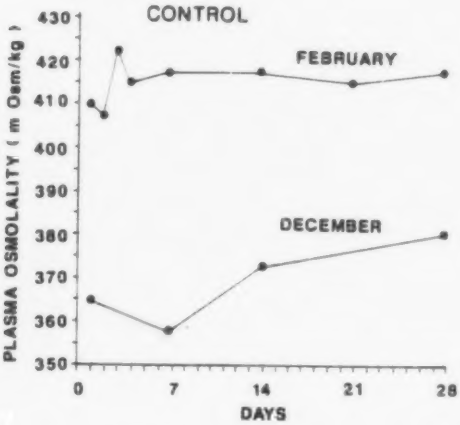


Fig. 5. Temporal changes in plasma osmolality in Atlantic cod transferred from stock tanks at 26-28 g/L salinity to experimental tanks also at 26-28 g/L salinity (control) in December and February. Temperature averaged 4.4°C in December and 1.2°C in February.

Plasma osmotic concentrations 28 d after transfer were significantly higher in February than in December (Table 1; Fig. 6). The analysis of variance revealed no interaction between month of transfer and salinity for salinities from 14-28 g/L. Month of transfer was highly significant, with cod transferred in February having much higher osmotic concentrations than cod transferred in December. For cod transferred to 7 g/L salinity, there was also a significant difference between the December and February experiments (Mann-Whitney's U-test, $p < .05$), although this difference amounted to only 5 mOsm/kg (Fig. 6).

No explanation can be given at this point to account for such a difference in osmotic concentrations between December and February. Two points should be made: 1) differences between months decrease at lower salinities (5 mOsm/kg at 7 g/L and 38 at 26-28 g/L), and 2) this result cannot be ascribed entirely to changes in sodium and chloride concentrations. Lower osmotic concentrations

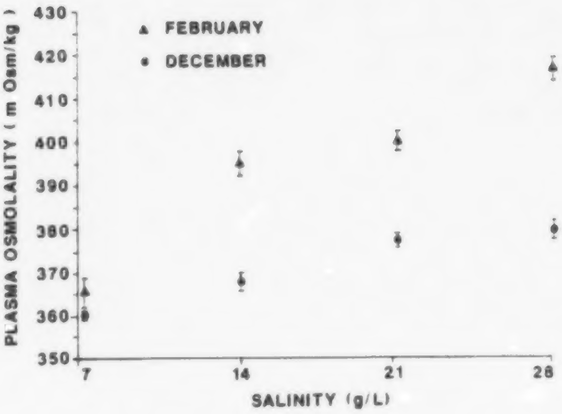


**Fig. 6. Plasma osmolality in Atlantic cod transferred directly to waters of three different salinities after 28 d in December and February. Controls were held in 26-28 g/L salinity. Temperature averaged 4.4°C (control) to 5.4°C (7 g/L) in December and 1.2°C (control) to 2.5°C (7 g/L) in February. Standard deviations are indicated as vertical bars.**

Table 1. Means and standard deviations of plasma solutes measured in Atlantic cod 28 d after they were directly transferred to waters of three different salinities in December and February. Controls were held in 26-28 g/L salinity. Temperature averaged 4.4°C (control) to 5.4°C (7 g/L) in December and 1.2°C (control) to 2.5°C (7 g/L) in February. Statistical tests were carried out for osmolality values only; values sharing the same number are not significantly different (SNK test, probability level 5%).

| | | | | | |
|---|---|---|---|---|---|
| | | December | | | |
| Salinity | (g/L) | 7 | 14 | 21 | 26 |
| Sodium | (mEq/L) | 175±1 | 177±1 | 178±7 | 183±5 |
| Chloride | (mEq/L) | 158±2 | 161±2 | 164±7 | 168±3 |
| Osmolality | (mOsm/kg) | $360\pm3^{1}$ | $367\pm8^{2}$ | $376\pm6^{3}$ | $380\pm8^{3}$ |
| | | February | | | |
| Salinity | (g/L) | 7 | 14 | 21 | 28 |
| Sodium | (mEq/L) | 177±8 | 188±3 | 191±2 | 196±2 |
| Chloride | (mEq/L) | 159±7 | 170±3 | 169±2 | 175±3 |
| Osmolality | (mOsm/kg) | $365\pm14^{1}$ | $394\pm11^{2}$ | $401\pm8^{2}$ | $418\pm10^{3}$ |

at lower salinities are to be expected and a decreased performance at lower temperatures would explain our first observation. The second observation may be unrelated to osmoregulatory performance. Indeed, the increase in osmotic concentration was much larger than increases in sodium and chloride concentrations alone in controls. Possibly, this increase in plasma osmotic concentration is related to the seasonal occurrence of the cod's mechanism of cold resistance. Cold resistance in cod is brought about by the synthesis of antifreeze proteins (Fletcher 1977; Fletcher et al. 1982; Hew et al. 1981). Those would not alter substantially the osmotic properties of the cod's plasma. However, because these molecules decrease the freezing point of plasma, they may have resulted in an overestimation of osmotic concentrations as measured with a freezing point osmometer (G.L. Fletcher, pers. comm.).

Following transfer to low salinities, plasma osmotic concentrations decreased significantly. Plasma osmotic concentrations followed changes in water salinity so that cod in the lowest salinity (7 g/L) had the lowest concentrations as measured on the 28th day. The analysis of variance confirmed that significant differences occurred between treatments at day 28. Tests made *a posteriori* included the 7 g/L salinity treatment since variances were homogeneous within a month of transfer. The 7 g/L treatment was found to differ significantly from the other two treatments and from the control, both in December and February (Table 1). Cod in the 14 g/L treatment also differed significantly from the controls. However, cod in the 21 g/L treatment did not differ significantly from the controls in December and from cod in the 14 g/L treatment in February. Results obtained in February, as mentioned earlier, may be an indication that cod in hypo-osmotic waters may not be able to survive at very low temperatures.

Rearing Atlantic cod under estuarine conditions thus seems feasible under the sole criterion of tolerance to low salinity. Direct transfer to fresh water killed cod over a short period, but cod resisted other treatments, including hypo-osmotic conditions. Perturbations were rather limited in view of the fact that cod were not allowed to acclimate progressively. New equilibrium conditions were reached in each case, usually within a week. Furthermore, there was relatively little individual variation. These results suggest that the potential of cod to grow in brackish waters should now be assessed.

Similar experiments are in progress in our laboratory to assess osmoregulatory performance at higher environmental temperatures and at lower salinities (0-7 g/L). Measurements of stress indicators such as cortisol and glucose were also made in order to assess stress levels following transfer to low salinities (Dr. Céline Audet, Institut National de la Recherche Scientifique-Océanologie, Rimouski, Québec) and will contribute to a better assessment of the feasibility of rearing cod in estuarine conditions.

## REFERENCES

Davies, P. L., C. L. Hew, and G. L. Fletcher. 1988. Fish antifreeze proteins: physiology and evolutionary biology. Can. J. Zool. 66: 2611-2617.

Fletcher, G. L. 1977. Circannual cycles of blood plasma freezing point and Na⁺ and Cl⁻ concentrations in Newfoundland winter flounder (*Pseudopleuronectes americanus*): correlation with water temperature and photoperiod. Can. J. Zool. 55: 789-795.

Fletcher, G. L., D. Slaughter, and C. L. Hew. 1982. Seasonal changes in the plasma levels of glycoprotein antifreeze, Na⁺, Cl⁻, and glucose in Newfoundland Atlantic cod (*Gadus morhua*). Can. J. Zool. 60: 1851-1854.

Hew, C. L., D. Slaughter, G. L. Fletcher, and S. B. Joshi. 1981. Antifreeze glycoproteins in the plasma of Newfoundland Atlantic cod (*Gadus morhua*). Can. J. Zool. 59: 2186-2192.

Odense, P., A. Bordeleau, and R. Guilbault. 1966. Tolerance levels of cod (*Gadus morhua*) to low salinity. J. Fish. Res. Board Can. 23: 1465-1467.

Saunders, R. L. 1963. Respiration of Atlantic cod. J. Fish. Res. Board Can. 20: 373-386.

Saunders, R. L., B. C. Muise, and E. B. Henderson. 1975. Mortality of salmonids cultured at low temperature in sea water. Aquaculture 5: 243-252.

Wu, R. S. S., and N. Y. S. Woo. 1983. Tolerance of hypo-osmotic salinities in thirteen species of adult marine fish: implications for estuarine fish culture. Aquaculture 32: 175-181.

# ATTENDANCE LIST

## CANADA-NORWAY FINFISH AQUACULTURE WORKSHOP

September 11-14, 1989

Snorre Tilseth, Chief
Division of Aquaculture
Institute of Marine Research
P. O. Box 1870, Nordnes
5204 Bergen, Norway

Vilhelm Bjerknes
Norwegian Institute for Water Research
Breiviken 5
N-5035 Bergen, Norway

Ole J. Torrissen
Institute of Marine Research
Matre Aquaculture Research Station
N-5198 Matredal, Norway

Tom Hansen
Institute of Marine Research
Matre Aquaculture Research Station
N-5198 Matredal, Norway

Jens Chr. Holm
Institute of Marine Research
Div. of Aquaculture
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

Anders Mangor-Jensen
Institute of Marine Research
Div. of Aquaculture
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

Kjell Naas
Institute of Marine Research
Div. of Aquaculture
Austevoll Aquaculture Research Station
N-5392 Storebø, Norway

Karin Pittman
Dept. of Fisheries Biology
University of Bergen
P. O. Box 1839, Nordnes
5024 Bergen, Norway

Sigurd Stefansson
Dept. of Fisheries Biology
University of Bergen
P. O. Box 1839, Nordnes
5024 Bergen, Norway

Brit Hjeltnes
Institute of Marine Research
P. O. Box 1870, Nordnes
5024 Bergen, Norway

Terje Svåsand
Institute of Marine Research
P. O. Box 1870, Nordnes
5024 Bergen, Norway

Knut Jørstad
Institute of Marine Research
P. O. Box 1870, Nordnes
5024 Bergen, Norway

Åsmund Bjordal
Institute of Fishery Technology Research
P. O. Box 1964
5024 Bergen, Norway

Mike Sinclair, Director
Biological Sciences Branch
Dept. of Fisheries and Oceans
P. O. Box 550
Halifax, N. S. B3J 2S7 Canada

Robert H. Cook, Director
St. Andrews Biological Station
Dept. of Fisheries and Oceans
St. Andrews, N. B. E0G 2X0 Canada

Richard Saunders
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

Henrik Kreiberg
Dept. of Fisheries and Oceans
Pacific Biological Station
Nanaimo, B. C. V9R 5K6 Canada

John Cornick
Dept. of Fisheries and Oceans
P. O. Box 550
Halifax, N. S. B3J 2S7 Canada

Gilles Olivier
Dept. of Fisheries and Oceans
P. O. Box 550
Halifax, N. S. B3J 2S7 Canada

Santosh Lall
Dept. of Fisheries and Oceans
P. O. Box 550
Halifax, N. S. B3J 2S7 Canada

Gil Farmer
Dept. of Fisheries and Oceans
P. O. Box 550
Halifax, N. S. B3J 2S7 Canada

Ken Waiwood
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

Richard Peterson
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

James Duston
c/o Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

Arnold M. Sutterlin
Bay D'Espoir Aquaculture
P. O. Box 189
St. Albans, Nfld. A0H 2E0 Canada

Jean-Denis Dutil
Dept. of Fisheries and Oceans
P. O. Box 1000, 850 Route de la Mer
Saint-Flavie, P. Q. G0J 2L0 Canada

Jean Munro
Dept. of Fisheries and Oceans
P. O. Box 1000, 850 Route de la Mer
Saint-Flavie, P. Q. G0J 2L0 Canada

Gerry Friars
SGRP, Atlantic Salmon Federation
P. O. Box 429
St. Andrews, N. B. E0G 2X0 Canada

David Wildish
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

John Bailey
SRGP, Atlantic Salmon Federation
P. O. Box 429
St. Andrews, N. B. E0G 2X0 Canada

Brigette de March
DFO, Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6 Canada

Greg Goff
Marine Science Research Lab
Memorial Univ. of Nfld.
St. John's, Nfld. A1C 5S7 Canada

Brian Glebe
Huntsman Marine Science Centre
St. Andrews, N. B. E0G 2X0 Canada

Larry Crim
Marine Science Research Lab
Memorial Univ. of Nfld.
St. John's, Nfld. A1C 5S7 Canada

Jennifer Martin
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B. E0G 2X0 Canada

Murray Hill, Supervisor
Inland Fisheries
P. O. Box 700
Pictou, N. S. B0K 1H0 Canada

Brian Rogers
General Manager
Sea Farm Canada
P. O. Box 2030
Saint John, N. B. E2L 3T5 Canada

Julie Delabbio
N. B. Community College
St. Andrews, N. B. E0G 2X0 Canada

Kevin Davidson
Science Br., Fisheries and Oceans
P. O. Box 5030
Moncton, N. B. E1C 9B6 Canada

Randy Penny
Dept. of Fisheries and Oceans
P. O. Box 5667
St. John's, Nfld. A1C 5X1 Canada

Gilles Boeuf
IFREMER
Centre de Brest
B.P. 70
29263 Plouzane, France

Debbie J. Martin-Robichaud
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0  Canada

Blythe Chang
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0  Canada

Mark Stewart
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0  Canada

Ken Howes
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0  Canada

Derek Knox
Connors Brothers
Aquaculture Division
Blacks Harbour, N. B.  E0G 1H0  Canada

Paul Harmon
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0  Canada

Maria-I. Buzeta
Dept. of Fisheries and Oceans
Biological Station
St. Andrews, N. B.  E0G 2X0

Linda Leblanc
Institute Maurice-Lamontagne
850 Route de la Mer
Mont-Joli, P. Q.  G5H 3Z4  Canada